PAPER 1 – LITERATURE REVIEW

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I. INTRODUCTION

Cotton has been used by humans for approximately 5,000 years. Archaeologists have found evidence that cotton has been used to produce garments in Africa, Pakistan and the Americas since 2500 BC (Stephens, 1975). Cultivated cotton produced today belongs to the genus *Gossypium* which consists of over 50 species. The majority of cotton produced worldwide today is *Gossypium hirsutum*, commonly known as upland cotton. There are 3 other species commercially produced including *G. barbadense* or Pima cotton and two Asian species, *G. herbaceum* and *G. arboreum*. These four species are commercially grown for the production of lint which can be spun into yarn (Applequist *et al.*, 2001).

Cotton production is an important industry worldwide, supplying the textile industry with raw fibre for the manufacturing of garments. Pressure from synthetic fibres has seen the industry become aware of the need for producing high yields of quality fibre in the most efficient manner. Precise management practices including fertiliser application and ground preparation play significant roles in accomplishing a superior product. Knowledge and experience of interactions between climate, plants, soils and microorganisms is needed to improve the efficiency and sustainability of cotton production.

II. AUSTRALIAN COTTON PRODUCTION

The Australian cotton industry plays an important role in the agricultural sector. Australia exports 95% of cotton produced generating approximately $1 billion per year in export revenue (Cotton Australia, 2006). Australia produces high quality cotton which fetches premium prices from overseas spinners. Australia ranks as the third largest exporter behind the U.S.A and Uzbekistan (Cotton Australia, 2006). The Australian cotton industry provides many employment opportunities due to its extensive production system and processing requirements.

In Australia, cotton has been grown commercially for about 35 years and there has been a significant expansion in production and quality. In 1975, cotton production reached 87,000 bales compared to the record yields of 2001 when 3.4 million bales were produced (Cotton Australia, 2006). About 70% of Australia's cotton is produced in NSW with the remainder grown in Queensland. Generally, cotton farms are run as family businesses with the

exception of a few large scale corporate operations. Cotton farms typically range in size from 500 to 2,000 ha (Cotton Australia, 2006).

Traditionally, cotton has been grown under irrigation on self mulching vertosol soils, common to the river valleys of the northern NSW and Queensland grain belt. Cotton farms are generally situated close to rivers for access to irrigation water. Bores are also a source of irrigation water. Producers need water licences to receive allocations for their irrigation needs. Cotton is grown as a summer crop in Australia and is planted from September to October in Australia (Constable, 1976). Soil temperature and the likelihood of frost play an important role in determining the date of planting. Cotton is a high value crop and requires large amounts of inputs. Development of infrastructure in cotton fields is required for irrigation purposes and water storages, channels, pumping stations, head ditches, tail drains and the gradient of fields are some of the development costs involved for the production of cotton.

(1) CROP DEVELOPMENT

Gossypium hirsutum has an indeterminate growth habit which needs to be managed to produce lint in one growing season. Cotton development can be divided into 5 growth stages: germination and emergence, seedling establishment, leaf area and canopy development, flowering and boll development, and maturation (Oosterhuis, 1990).

(*a*) GERMINATION AND EMERGENCE

Planting of cotton seed should occur when the soil temperature reaches 14^{o}C at a depth of 10 cm for at least three days. Cotton seeds require more oxygen for germination than other plants such as maize, wheat and rice (Eaton, 1955). The seeds also require relatively high moisture and high soil temperatures for successful germination. Cotton seeds imbibe water rapidly, generally taking 36 to 48 h (Hearn & Constable, 1984; Wanjura & Buxton, 1972) and begins when water enters through the chalaza and later through the whole testa entering the embryo (Figure 1) (Oosterhuis, 1990).

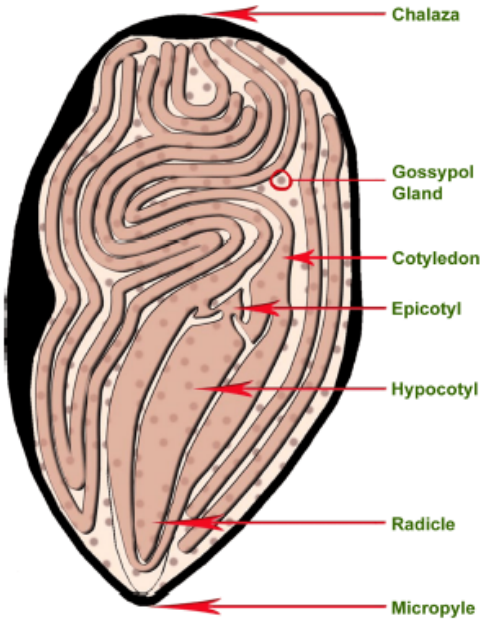

Figure 1: Cross section of a cottonseed (Ritchie *et al.*, 2004)

The seed swells and splits, allowing the radicle to emerge. The cotyledons are pushed above the soil after germination by the elongation of the hypocotyl (Eaton, 1955). Compaction or crusting of the soil surface can restrict the emergence of the cotyledons. In favourable conditions, cotton seedlings can emerge in 4 to 14 days after planting (Ritchie *et al.*, 2004).

(*b*) SEEDLING ESTABLISHMENT

The cotyledons grow rapidly producing carbohydrates for 10 to 12 days before the first true leaf appears. Early development of the cotton plant focuses on root development, resulting in a relatively slow growth rate of the above ground portion (Oosterhuis, 1990). The radicle or primary root can reach a significant depth of up to 30 cm, before the cotyledons emerge (Ritchie *et al.*, 2004). The *Gossypium* genus is renowned for having strong taproots which can penetrate up to 3 m in a suitable soil (Hearn *et al.*, 1984). Lateral roots are also produced which can extend up to 1 m. The root activity declines as bolls develop, requiring more carbohydrates that are redirected from the roots (Oosterhuis, 1990).

Seedling development is an important stage in the growth cycle of a cotton plant. A good stand of healthy plants is important. The seedling faces many challenges and is vulnerable to soil borne fungi such as Pythium (*Pythium aphanidermatum*), Rhizoctonia or damping off (*Rhizoctonia solani*) and Thielaviopsis (*Thielaviopsis basicola*). Ensuring that the seeds are

planted at a suitable depth of 3 to 5 cm and at a rate of 10 to 13 plants per m (Cotton Seed Distributors, 2004) will minimise the risk of seedling damage or loss by fungal pathogens. Other factors that influence healthy vigorous seedlings are soil temperature, moisture availability and compaction.

The main stem of a cotton plant develops from elongation of the terminal bud (Oosterhuis, 1990). The main stem consists of nodes and internodes (Figure 2) and does not terminate in an inflorescence like sorghum or wheat (Hearn *et al.*, 1984). A node can be produced every 2 to 4 days if conditions are favourable. The length of the internodes and number of nodes are determined by environmental factors and genetics (Oosterhuis, 1990). The main stem is monopodial or vegetative in supporting true leaves and sympodial or fruiting branches (Ritchie *et al.*, 2004). Branches develop from buds located at a node. A vegetative branch may develop which is structurally the same as the main stem. Vegetative branches generally occur if the terminal on the main stem has been damaged (Oosterhuis, 1990).

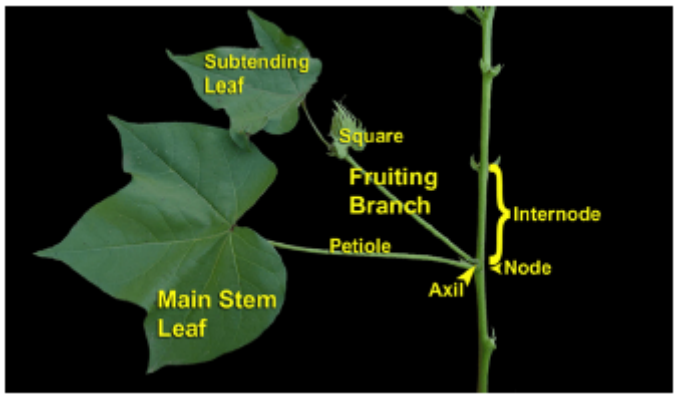

Figure 2: Growth of a fruiting branch of a cotton plant (Ritchie *et al.*, 2004)

Fruiting branches develop from nodes on the main stem and other vegetative branches (Figure 2). When a fruiting branch develops, a leaf and a flower bud or square is also produced at the same node (Oosterhuis, 1990). The elongation of the internode behind the square enables the fruiting branch to extend away from the main stem. The development of the fruiting branch terminates in a square or fruiting position (Oosterhuis, 1990). This leads to a second leaf and fruiting bud to develop at the axil of the first leaf and the process continues with extension of the internode (Figure 2). It is common to see a fruiting branch with three to four fruiting positions. The fruiting pattern of a cotton plant can be described as spiralling outward and upward in a 3/8 phyllotaxy (Figure 3) (Ritchie *et al.*, 2004).

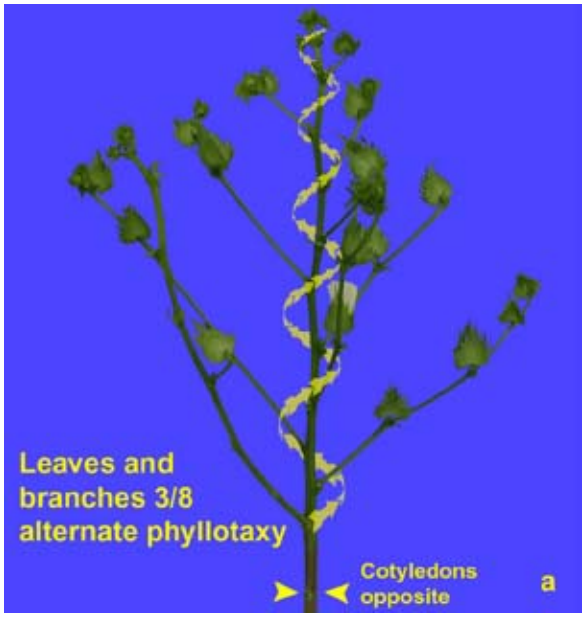

Figure 3: The leaves and branches of cotton in a 3/8 alternate phyllotaxy (Ritchie *et al.*, 2004).

Cotyledons, prophylls and true leaves are the three types of leaves present on a cotton plant. The cotyledons are the first leaves to appear. They are kidney-shaped and paired or opposite on the main stem. The prophylls are the first leaves to develop on a branch (Oosterhuis, 1990). The prophylls are small and lack a petiole. The true leaves of a cotton plant vary in size. On average, true leaves are approximately 10 to 15 cm wide when developed. Cotton leaves reach their full size in about 2 to 3 weeks without stress factors (Hearn *et al.*, 1984). Main stem leaves are approximately twice the size of leaves found on the fruiting branches (Hearn *et al.*, 1984). True leaves can appear entire or deeply lobed (Oosterhuis, 1990). Cotton leaves have a thick waxy cuticle and small hairs on the surface. Similar to branches, cotton leaves are arranged in a spiral configuration up the main stem (Oosterhuis, 1990). The cotton leaf supports the growth of its nearest vegetative parts (Hearn *et al.*, 1984).

(*c*) LEAF AREA AND CANOPY DEVELOPMENT

Vegetative growth provides support for later fruit development (Oosterhuis, 1990). The development of the canopy is also important for maximising the amount of light intercepted for photosynthesis. The blade of the cotton leaf follows the sun throughout the day (Hearn *et al.*, 1984). Canopy closure is an effective tool for the suppression of weeds and the minimisation of water loss from the soil (Oosterhuis, 1990). The Leaf Area Index (LAI) is

the measurement of the developing canopy. The optimum LAI occurs 3 to 5 weeks after flowering (Hearn *et al.*, 1984). Vegetative growth must be managed appropriately to maximise yield. Producers often apply Pix® (mepiquat chloride), a chemical which suppresses the vegetative growth of the plant and promotes reproductive or fruit growth (Munk et al., 1998).

(*d*) FLOWERING AND BOLL DEVELOPMENT

Reproductive development occurs approximately 4 to 5 weeks after planting. At this time the floral buds are forming in the upper part of the plant (Oosterhuis, 1990). These floral buds are known commonly as squares. The square consists of 3 bracts which purposely cover and protect the reproductive parts. Squares and young bolls are often shed by the cotton plant. Shedding is a natural occurrence which is aided by environmental factors such as water stress, overcast conditions and insect damage (Figure 4) (Oosterhuis, 1990).

Figure 4: examples of square or boll shedding of a cotton plant (Ritchie *et al.*, 2004)

The first flower appears from the square about 7 to 8 weeks after planting. The flowers towards the bottom of the plant open first. The first flower will open from the first fruiting position on the first fruiting branch. It takes about 3 days for the next flower to open on the same position on the next fruiting branch (Oosterhuis, 1990). The next flower on the same fruiting branch will open 6 days after the flower on the previous fruiting position (Figure 5).

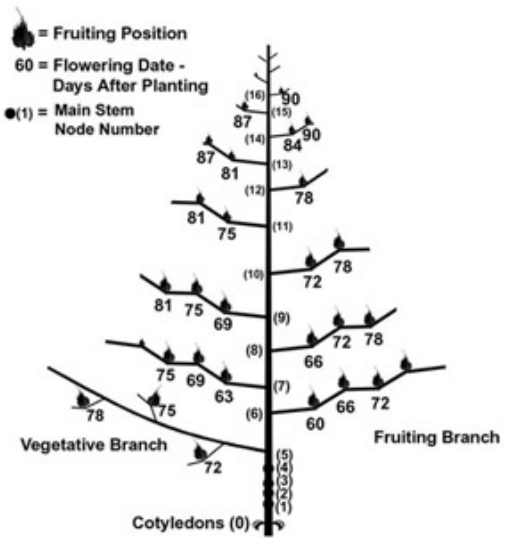

Figure 5: Fruiting pattern and structure of a cotton plant (Albers, 1993)

The cotton flower posses male and female reproductive parts. The stamen and anther are male and the stigma, stylet and ovary are female (Figure 6) (Ritchie *et al.*, 2004). Flowers open at dawn and are generally fertilised within a few hours (Oosterhuis, 1990). Cotton flowers are usually self pollinated (Hearn *et al.*, 1984) when pollen falls from the anther onto the sticky surface of the stigma. Insects, particularly bees, can increase the amount of cross pollination. The white or cream flower begins to turn pink after pollination has occurred and is shed a few days later. The fertilised ovules develop into hard coated seeds which produce lint (Hearn *et al.*, 1984).

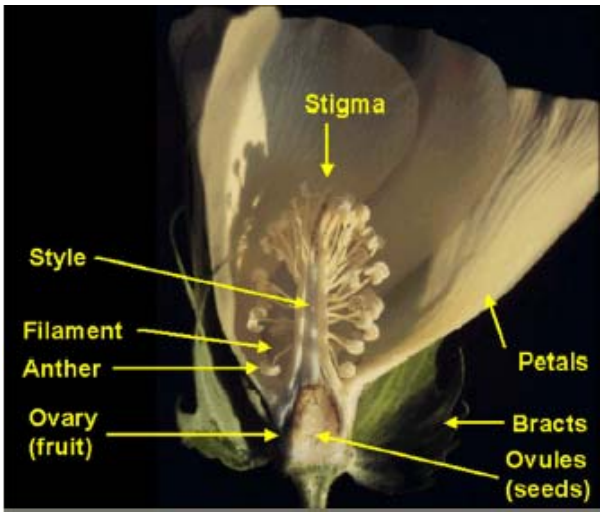

Figure 6: Cross section of a cotton flower (Ritchie *et al.*, 2004)

(*e*) MATURATION

Boll development is essentially the progression of the cotton fibre development. Lint develops from the epidermal cells of the seed coat (Hearn *et al.*, 1984). Fibre development begins with initiation, where the epidermal cell expands (Seagull *et al.*, 2000). Elongation is the second stage which refers to the expansion phase of fibre development, followed by secondary wall synthesis, the major phase of fibre growth (Ritchie *et al.*, 2004). Secondary wall synthesis explains the process of depositing cellulose after elongation has occurred, strengthening the fibre whilst developing its thickness or micronaire (Seagull *et al*., 2000). Maturation is the final stage of fibre development. This phase begins when the bolls open and the metabolically inactive cotton fibre dries out (Seagull *et al*., 2000).

(2) COTTON GROWING AREAS

The majority of Australian cotton production occurs in NSW. The production area stretches from the Macintyre River on the Queensland border through to the Gwydir, Namoi and Macquarie River valleys. Some production occurs in the Lachlan and Murrumbidgee valleys in the south west of NSW and along the Darling and Barwon Rivers in the west of the state (Cotton Australia, 2006). Southern Queensland produces the majority of Queensland cotton, including the Macintyre valley, and St George and Darling Downs areas. Cotton is also produced in central Queensland around Emerald and Theodore (Figure 7) (Cotton Australia, 2006). Cotton has been produced in northern Australia in previous years with early success however high insect pressure and nutrient deficient soils halted production. Stage II of a project at Kununurra, in the Kimberly region of northern Western Australia, has shown promising results for cotton as a winter or dry season crop (Duggan & Ryan, 2004). The abundant amount of water available from Lake Argyle on the Ord River supports a small tropical fruit industry. A further 45,000 Ha is proposed for cropping, cotton has been tagged as an ideal crop for such an area (Duggan *et al.*, 2004). Fertiliser and pest management are the key issues impeding full scale production at the present time.

Figure 7: Cotton production areas of Australia (McKenzie, 1998)

(3) TEMPERATURE AND TIME OF PLANTING

Temperature is a very important factor controlling the growth and development of cotton. Temperature influences the phenological, morphological, physiological and biochemical processes of cotton (Liakatas *et al.*, 1998; Wall *et al.*, 1994). Cotton growth is controlled by temperature and requires a minimum number of day degrees for optimal crop development. Being a tropical crop, *Gossypium hirsutum* thrives on warm daily temperatures that have an effect on yield and cotton fibre properties such as strength fineness and micronaire (Liakatas *et al.*, 1998).

Planting time may be delayed if conditions are wet and cold (Constable, 1976). Some crops are affected by disease or hail and have to be replanted to improve the stand. In cooler cotton growing areas of Australia such as Gunnedah, the planting date is crucial as planting too early or too late can result in yield reductions or poor fibre quality. Reduction in fibre quality is associated with late planting as the bolls may develop through the cooler and wetter autumn months (Constable, 1976). In hotter areas of Australia such as Emerald, temperature at planting time is less of an issue (Cotton Seed Distributors, 2004).

Cool soil temperatures will reduce seedling vigour and increase susceptibility to diseases and insect damage, whereas warmer soil temperatures promotes better growth of plants and leaves for later reproductive development (Cotton Seed Distributors, 2004).

III. SOILS

In Australia, cotton is mainly grown on black or grey vertosols and some chromosols and sodosols in the Macquarie Valley (McKenzie, 1998). Vertosols are self mulching clays that have resulted from alluvium or aeolian deposits (Isbell, 1996). The Black clays of Emerald are formed directly on basalt (McKenzie, 1998). Cracks often extend to the surface and slickenside or lenticular peds are usually found at depth (Isbell, 1996).They have a uniform profile and are classified as heavy clays, possessing greater than 35 % clay throughout the profile (Table 1). Australia has a diverse array of vertosols with difference in colours often associated dispersion (Isbell, 1996). Black vertosols contain the clay mineral smectite, while other sub-orders contain illite and kaolin (Isbell, 1996).

Table 1: Typical characteristics of a vertosol profile (Dalal *et al.*, 1995)

Soil depth (cm)	Bulk density (Mg/m^3)	pH	Sand	Silt	Clay (%)	Organic C	Total N	C/N
0–10	1.24	8.6	27	17	56	0.74	0.072	10.3
10–20	1.27	8.9	27	16	57	0.63	0.056	11.3
20–30	1.28	9.0	28	15	57	0.59	0.046	12.8
30–60	1.36	9.0	25	16	59	0.51	0.036	14.2
60–90	1.42	7.7	20	17	63	0.42	0.026	16.2
90–120	1.43	5.3	19	16	65	0.37	0.020	18.5
120–150	1.45	4.9	19	15	66	0.33	0.021	15.7

Vertosols are often associated with gilgais and are found extensively in Qld, NSW and NT, generally on large floodplains (Isbell, 1996). The most common landuse for vertosols are grazing, cereal, and rice and cotton production.

The high clay content of vertosols allows the soil to have a high water holding capacity. Vertosols have a shrink-swell nature which is important for irrigation management (Vervoort *et al.*, 2003) and for describing the proliferation of plant roots. Large values of plant available water capacity (PAWC) are characteristic of most cotton growing soils (McKenzie, 1998). This is can allow a longer interval between irrigations and in the case of dry-land crops it delays the inception of water stress.

The shrink-swell characteristic of vertosols can give rise to the potential problem of compaction by machinery (Isbell, 1996). The pH of vertosols is generally alkaline, often having a pH of 7 or more. The pH tends to increase in alkalinity down the profile (Isbell, 1996). Vertosols are generally fertile with organic matter ranging from 1% to 2.3% and relatively high calcium and phosphorus levels. The clay minerals of a vertosol are negatively charged, attracting the positively charged cations which are often essential nutrients required for plant growth (McKenzie, 1998). The ability to attract cations is known as the Cation Exchange Capacity (CEC). Vertosols are known to have high CEC's due to high clay content. Organic is also important in soils CEC as humus particles are often positively charged and can attract and store the negatively charged ions such as nitrate, phosphate and sulphate (McKenzie, 1998).

An ideal soil for cotton production needs to have a good draining surface with negligible drainage beneath the root zone (McKenzie, 1998). Compaction needs to be minimised, traffic farming coinciding with satellite guidance systems is a method used to reduce compaction and smearing of the soil. The appropriate soil must have high PAWC, not be too saline and possess adequate nutrients (McKenzie, 1998). Vertosols boast a relatively neutral pH and most of the attributes above. These characteristics along with suitable management practices make them a desirable soil for cotton production.

(1) SOIL LIMITATIONS

Traditional farming practices have had an adverse effect on soil health and fertility. Conventional tillage including disc and chisel ploughing on a regular basis along with deep ripping and laser levelling every few years, has led to the decline of soil structure and chemical fertility (Hulugalle *et al.*, 1999b). Poor cropping practices such as crop monocultures have led to the degradation of soil fertility. Farmers are increasingly becoming aware of how important soil health is for their production systems. Minimum or zero tillage practices have been implemented across much of Australia's grain belt. This is one method farmers have developed to maintain the productive capability of the soil (Benjamin *et al.*, 2003). Minimum tillage incorporates the preservation of crop residues which limit evaporation, increase soil organic matter, prevent erosion and increase infiltration rates (Guerif *et al.*, 2001). Historically cotton was produced as a monoculture with heavy reliance

on tillage and chemical fertilisers. In the Namoi Valley, continuous cropping has caused soil health and structural problems, however the use of rotations can reverse the degrading trend (Cooper, 1999).

The major rotational crop employed by Australian cotton producers is wheat (*Triticum aestivum* L.) and sorghum (*Sorghum bicolor* L.). Legumes such as chickpeas (*Cicer arietinum* L.) and field peas (*Pisum sativum* L.) are other alternative rotational crops (Cooper, 1999). Using a legume in a crop rotation is beneficial as nitrogen is fixed, reducing reliance on fertiliser. The use of crop rotations are said to improve soil structure, reduce incidence of disease and weeds and increase cotton yield (Hulugalle *et al.*, 1999a). Wheat has a fibrous root system which can dry out the profile improving soil structure (Constable, 2004) and is harvested 9 months prior to cotton planting allowing nitrogen mineralisation to occur over the fallow (Constable, 2004; Rochester *et al.*, 1991). In Australia the average rotation is two years of cotton proceeded by one year of wheat (Cooper, 1999).

IV. YIELD

Producers of commercially important crops strive for high quality and yield. Cotton farmers are paid on a per 227 kg bale basis for the fibre that is produced. Like cereal grain producers, cotton farmers benefit from producing as much lint or yield as possible. Prices received can be reduced if certain lint quality attributes are not met. Breeding has been a major factor determining yield in the Australian cotton industry. New varieties and enhanced management practices have helped increase yields from 110,000 bales in 1975 to the record breaking yields of 3.4 million bales in 2001 (Cotton Australia, 2006).

(1) YIELD COMPONENTS

Cotton breeding still focuses primarily on yield (Hearn *et al.*, 1984). New varieties are continuously being produced which contribute to higher yields. Cotton is similar to most of the commercially grown crops in that one of the major yield components is the seed or the number of seeds produced. There are genetic and structural limitations of a plant as to how many seeds are produced (Koide, 2000). However many external influences such as climate and nutrient resources are usually the determinants. There have been many approaches to what constitutes yield. The number of bolls/m^2 is suggested to be the most important contributor to lint yield, followed by seeds/boll and lint/seed (Worley *et al.*, 1974). Boll size

and the lint percentage are also physical attributes that determine the yield of cotton (Iqbal *et al.*, 2003; Worley *et al.*, 1974). Sympodial branches have a direct relation to yield as the branches support the bolls and ultimately the seed cotton (Iqbal *et al.*, 2003). Breeders utilise genetic information regarding these branches so that new varieties which produce more fruiting branches can be developed. The number of bolls and the percentage that are retained are also important components of yield. The number of bolls retained is influenced by management practices, environmental conditions and genotypes (Iqbal *et al.*, 2003). Understanding the genetics behind retention and breeding for this specific trait would make higher yields attainable. Research on boll set has revealed that there is definite variation between different cultivars of upland cotton (Iqbal *et al.*, 2003). Cotton breeders are continuously investigating methods of developing new varieties that will ultimately produce high yields with superior quality and will reduce input costs (Cheatham *et al.*, 2003).

There are complex relationships between cotton lint yield and the components which generate it. Genetics and environmental conditions play a huge role in determining which yield components contribute to yield (Worley *et al.*, 1974). There have been a number of models produced to illustrate the components of yield. An example of such an equation is that the number of seeds per ha and the weight of the fibre per seed are the components of lint yield (Lewis, 2001). Such a model can be written as follows:

Lint Yield = (Seeds/Hectare) x (Weight of Fibre/Seed)

Another equation that has been used for determining yield from yield components can be seen below (Worley *et al.*, 1974):

Lint Yield = Bolls/M^2 x Seeds/Boll x Lint/Seed

Different equations illustrating yield component are useful in describing how yield is attained. The yield of any crop can be broken down into yield components to determine how yield is attained. Determining these yield components is important for breeders who can place more emphasis on that particular component. In a lot of cases, data is the limiting factor regarding which equation can be used.

V. COTTON NUTRITION

Cotton is primarily grown on Vertosols which are naturally fertile. Vertosols generally contain high levels of calcium (Ca) and phosphorus (P) (Dalal *et al.*, 1995), which can be limited in availability depending on its form. The nutrients that are needed for plant growth are naturally present in the mineral form in soil solution. Most nutrients are pooled in soil organic matter. Continuously cropping the soil will accelerate the depletion of most essential nutrients. Applications of fertilisers or incorporating legumes in a crop rotation are some methods of replacing these nutrients. Effective management of crop nutrition requires knowledge of the cropping system to understand the sources of nutrients and facilitate appropriate decisions concerning fertiliser application. The major essential nutrients utilised by cotton plants can be separated into 2 categories; macronutrients (Table 2) and micronutrients.

Table 2: Macronutrients essential for plant growth and development

Macronutrients	**Source**	**Role**	**Reference**
Nitrogen (N)	Mineral N, Fertiliser N, Legume fixed N	Growth; synthesis of proteins	(Vance, 2001)
Phosphorus (P)	Soil solution P, Absorbed P, Mineral P, Organic P and P fertilisers	Energy transfer, DNA and RNA, regulates metabolism and stored energy in seeds.	(Schachtman *et al.*, 1998)
Potassium (K)	Soil solution K, Exchangeable K, Non-exchangeable available K, Mineral K and K fertilisers	Osmotic regulation and translocation of carbohydrates	(Spalding *et al.*, 1999)
Calcium (Ca)	Soil solution Ca, Exchangeable Ca, Mineral Ca and lime or gypsum	Essential for cell wall formation	(Bush, 1995)
Magnesium (Mg)	Soil solution Mg, Exchangeable Mg, Mineral Mg and Mg fertilisers	Constituent of chlorophyll, oil synthesis and N metabolism	(Constable *et al.*, 2001)
Sulfur (S)	Organic matter, Mineral S, S fertilisers and Soil solution S.	Constituent of proteins and compounds involved in photosynthesis	(Droux, 2004)

(1) PHOSPHORUS

P is one of the most important macronutrients essential for plant growth. After nitrogen, P is the most limiting element for plant growth (Vance, 2001). It is present in nucleic acids, phospholipids and ATP (Schachtman *et al.*, 1998). P is involved in two important transformations of energy in plants, being adenosine diphosphate (ADP) and adenosine triphosphate (ATP) (Penfold, 2000). P also plays a role in regulating metabolic pathways and certain enzyme reactions (Theodorou & Plaxton, 1993).

P is normally absorbed by roots and transferred to the leaves via the xylem. However in P deficient plants, P is often translocated from stores in older leaves to growing shoots, young leaves and roots (Schachtman *et al.*, 1998). The amount of P found in plants varies from 0.05 to 0.30% of dry weight (Vance, 2001), depending on species and conditions. P is an important element in late season crop nutrition. P is associated with premature senescence where waterlogging or unfavourable growing conditions limit the uptake of P, reduce P in the leaves, decreasing photosynthesis and metabolism causing senescence (Constable *et al.*, 2001).

Australian soils are much older than soils of the American and European continents. Certain soils may contain high amounts of P. However, it is often present in unavailable forms (Schachtman *et al.*, 1998). P is generally unavailable because of its ability to form insoluble complexes with cations and can be incorporated into organic matter by microbial action (Vance, 2001). Most crop plant species require nutrients throughout the whole growing cycle. The ability of plants to accumulate nitrogen is relatively high compared to nutrients such as P. Phosphorus is slowly released by soils into available forms, often causing depleted zones around the roots of crop species (Schachtman *et al.*, 1998). The availability of soil P to plants is influenced by the soil condition. The soil pH, arbuscular mycorrhizal fungi (AMF) and the presence of microorganisms affect the availability of P (McLachlan, 1980). Only 20 to 80% of phosphorus in soils is in the available form (Schachtman *et al.*, 1998). Soil P can be classified into three pools which include available P, labile P and poorly available P (Constable *et al.*, 2001). Phytic acid is the main constituent of available P, with the remainder being comprised of 170 mineral forms of P which make up the labile and poorly available fraction (Holford, 1997). Inorganic P (Pi) is the main source of available P. However Pi makes up less than 1% of total (Bolan, 1991).

Most Pi is absorbed onto the soil surface or precipitated as specific phosphates allowing acquisition by the root zone of the plant (Bolan, 1991).

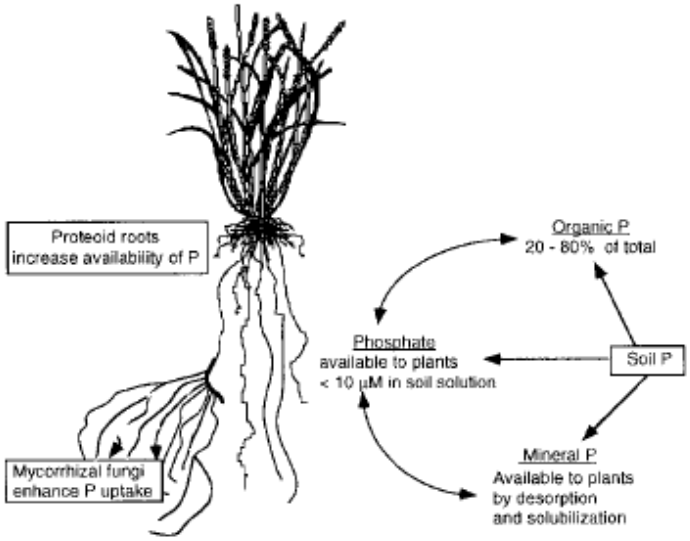

Figure 8: Plant acquisition of soil P (Schachtman *et al.*, 1998)

Plants have different modes of action for acquiring available P. Plant roots must come in contact with the nutrients either by root interception, mass flow or diffusion. P moves through the soil by diffusion whereas more soluble minerals such as potassium move readily by mass flow (Schachtman *et al.*, 1998). Diffusion rates of P in soil solution is generally very low because soil particles easily bind P (Wissuwa, 2003). Root hairs and AMF play an important role in nutrient uptake by the plant (Figure 8). The symbiotic fungi increases the effective surface of plant roots, enabling more soil to be exploited for nutrients (Schachtman *et al.*, 1998). Root hairs also increase the surface area of roots and the plant's ability to uptake nutrients (Dorahy *et al.*, 2004).

(*a*) PHOSPHORUS DEFICIENCY AND SYMPTOMS

The extent of P deficiency is the difference between the plant's demand for the nutrient and the availability of P in the soil, around the root zone (Koide, 2000). P deficiency is a major abiotic stress that can limit plant growth and crop productivity of commercially important crops throughout the world (Wissuwa, 2003). P deficiency is associated with old weathered soils and

areas of low fertiliser use; this can be more prevalent in developing areas where resources are limited (Wissuwa, 2003). P may be abundant in the soil but plants can not take up the organic form.

Plants have diverse responses to nutrient deficiency often due to inherent growth rates and the initial store of nutrients in the seed. Other influencing factors include genotype and environmental conditions (Atkinson, 1973). Studies have been performed on different plant species to identify responses to P deficiency. Small seeded species may be more prone to P deficiency than larger seeded plants due to their vigorous growth rates and exhaustion of seed reserves, which makes them more dependant on external nutrient supplies at an early stage (Atkinson, 1973).

The growth rate of P deficient plants is one of the most noticeable symptoms. Studies performed on *Spirodela* (Duckweed), have shown significant changes in growth rates after a plant with adequate P was transferred to a P deficient environment (Bieleski, 1968). The root/shoot ratio increases in a P deficient plant due to the growth of roots searching for nutrients and the reduction of shoot growth (Atkinson, 1973). P deficiency has a negative effect on the actual growth of the plant and the rate of leaf expansion (Singh *et al.*, 2006).

The major symptoms to watch for when identifying P deficiency is slow seedling emergence often resulting in stunted plants and a poor stand (Dorahy *et al.*, 2004). The appearance of dark green foliage and a purple tinge to the veins and petioles is another noticeable symptom of P deficiency (Incitec Pivot, 2003).Ultimately, any commercially important crop which suffers from a nutrient deficiency will incur some form of yield loss.

(*b*) PHOSPHORUS FERTILISER

Approximately 40% of the worlds arable land is limited by the availability of P for optimal plant growth, the most limited being the acidic soils of tropical regions (Vance, 2001). Australian cotton producers are becoming increasingly aware of nutrient deficiencies in soils suitable for cotton production. Fertilisers are used to provide nutrients to plants so that a maximum yield is achievable (Morel & Fardeau, 1990). In Australia, P fertiliser is usually applied prior to planting

(Dorahy *et al.*, 2004) and incorporated with primary tillage. A pre-plant broadcast application of P fertiliser increased cotton yield on soils which respond to additional phosphorus (Howard *et al.*, 2001). Phosphorus fertilisers typically used by Australian producers include; Single Superphosphate (Super), Triple Superphosphate (TSP), Monoammonium Phosphate (MAP) and Diammonium Phosphate (DAP). These fertilisers vary in constituents and amount of P from 8.8% in Superphosphate to 21.9% in MAP (Incitec Pivot, 2003). The most common form of P fertiliser is MAP as it forms a sightly acidic product and remains in solution longer than other fertilisers (Constable *et al.*, 2001).

Table 3: Soil tests commercially available for phosphorus.

Soil P test	**Method**	**Source**
Colwell bicarbonate P	Improved Olsen test, 16 hours extraction.	(Colwell, 1963)
Olsen P	P determined colorimetrically after 30 minutes, widely used, suited to alkaline soils	(Holford, 1997)
Hedley fractionation	Removes readily available P with extractants. Progresses to stable forms of P	(Guo *et al.*, 2000)
Acidic anionic extractants (numerous methods)	Integrate intensity and quantity of labile P. Gives critical P concentration	(Holford, 1997)
Multi-nutrient tests		
Mehlich- 1	Not suited for alkaline soils, 1:5 soil/extractant ratio, colorimetry	(Howard *et al.*, 2001)
Kelowna soil P test	Acidic anionic extractant	(Dorahy *et al.*, 2004)
Nuclear magnetic Resonance (NMR)	Spectroscopy to measure inorganic P.	(Dorahy *et al.*, 2004)

Commercially available soil nutrient (P) tests vary in time taken (Table 3). For example, Olsen P takes approximately 30 minutes compared to approximately 16 hours for Colwell P. The cost would obviously vary depending on method and nutrient(s) being identified. Fertiliser

recommendations are generally based on soil and plant tissue tests. There are a number of soil tests available for measuring the soil P (Table 3). However, Dorahy *et al.* (2004) suggested that none of the tests have been calibrated for Australian soils but are based on overseas experience on different soil types. The soil and plant tissue tests provide results from which suitable recommendations can be made (Table 4). There are many suggested critical limits for when fertiliser should be applied. Each crop has a different requirement for P. There are various critical limits for other crops such as wheat which will limit productivity if the Colwell-P is less than 30mg/kg (Colwell, 1963).

Table 4: Critical levels of P for cotton production suggested by different researchers

Method (see table 4)	**Critical limit of P in soil**	**Reference**
Bicarbonate extraction	10–20mg/kg	(Hibberd *et al.*, 1990; McKenzie, 1998)
Olsen-P	5-10mg/kg	(Dorahy *et al.*, 2004)
Mehlich-1	4-10mg/kg	(Howard *et al.*, 2001)
Acidic anionic extractants	<11mg/kg	(Bronson *et al.*, 2001)

There is a growing interest in plant tissue testing as a technique to measure the nutrient status of a plant. The youngest mature leaf is the best part of the plant for sampling as it should give a clear representation of what nutrients are utilised by the plant. If the crop exhibits P deficiency symptoms, it will not usually respond to direct application of P fertiliser (Dorahy *et al.*, 2004).
The response of plants to P fertiliser depends on the availability of P in the soil. When P fertiliser is applied to soils with low extractable P, the plant responds to as little as 29Kg ha of P compared to no response when fertiliser is applied to soils with high extractable P (Howard *et al.*, 2001). The application of high rates of P to a cotton system improved water relations when a plant is under water stress (Singh *et al.*, 2006).

A grain crop of 7 tonnes ha could require as much as 120kg ha P to generate such a yield (Bieleski, 1973; Vance, 2001). Of the amount of fertiliser applied, approximately 20% is

removed by the crop during its growing cycle (Vance, 2001). The accumulation of P fertiliser in agricultural soils has evoked criticism towards the industry. P loading is the term used to describe the effect of applying surplus fertiliser to agricultural lands. Runoff from the P-loaded soils was suggested to cause eutrophication and hypoxia of water sources in close proximity to these soils (Vance, 2001). This concern is accompanied by the fact that the P reserves could be depleted in 60 to 80 years (Vance, 2001). These limitations relating to P fertiliser application are some reasons why strategies are needed to improve plant acquisition of P.

Alternative practices have been suggested to reduce the reliance upon fertilisers. These strategies vary from the improvement of genotypes by plant breeders and management practices that can be implemented to improve the availability of P. Plant biologists are discovering mechanisms in plants which enable them to accumulate P more efficiently. Expanding root growth and root hair development are examples of strategies that will lead to better P uptake (Vance, 2001). Producers can increase the availability of P by including legumes into their crop rotations or even as a companion crop to certain cereals. Legumes, especially white lupin (*Lupinus albus*) can increase P availability (Vance, 2001). The root system of white lupin is will exude high concentrations of organic acids and acid phosphatase (Johnson *et al.*, 1996). The release of acids by the roots solubilise the inorganic P and the acid phosphatase can readily solubilise P that is organically bound (Vance, 2001). The cluster root formation of white lupin is an adaptation to low P as it increases the availability of P to the plant. Other strategies to overcome P deficiency include citrate exudation and synthesis in plant cells (Vance, 2001). Again the release of this chemical breaks down insoluble P to forms that are available to the plant.

The use of variable-rate fertilisation is an emerging management practice which could optimise the amount of fertilisers used and hence, reduce production costs for the producer. However, work performed on variable-rate application showed small responses compared to traditional blanket-rate application (Bronson *et al.*, 2001).

VI. ARBUSCULAR MYCORRHIZAL FUNGI

Beneath the soil surface surrounding plant roots is a region of microbial activity (Gerdemann, 1968). Many microorganisms are parasitic and infect roots often causing disease. However some highly specialised microbes form associations with plant roots without affecting the host (Gerdemann, 1968). These microorganisms are called arbuscular mycorrhizal fungi (AMF) and form a symbiotic relationship with the host, obtaining food whilst absorbing nutrients beneficial for the host. They are found in naturally occurring and disturbed soils associated with agriculture (Bolan, 1991). AMF belong to the Zygomycota family consisting of one order Glomales, and 6 genera where 149 species have been classified (Harrison, 1999). Many commercially important crops are colonised by AMF (Ryan & Angus, 2003).

AMF can enhance plant growth and uptake of nutrients (Price *et al.*, 1989). AMF are the most common and widespread fungi that are involved with plants at a symbiotic level (Smith *et al.*, 2001). The presence of AMF can also influence some root pathogens (Ryan *et al.*, 2003). One particular species of AMF, *Glomus mosseae* has shown to decrease severity of *Fusarium vasinfectum*, commonly known as Fusarium Blight (Zhengjia & Xiangdong, 1991). Other interactions between AMF and the cropping system environment include plant and water relations and suggestions of improvement in soil health (Ryan *et al.*, 2003).

(1) INFECTION AND SYMBIOSIS

AMF colonisation begins with primary infection caused by propagules found in the soil (Nehl *et al.*, 1998). A pattern of infection has been suggested for AMF on annual plants (Pattinson & McGee, 1997). There are three phases including (1) a lag phase followed by the primary infection, which is due to the germination of the propagules and the initial infection of the root, (2) the log phase which is the rapid secondary spread and (3) finally reaching a constant relationship between root and AMF (Pattinson *et al.*, 1997). Initiation is relatively slow and depends on the amount of propagules present in the soil. Secondary spread is much quicker and usually begins approximately 10 days after initial infection (McGee *et al.*, 1999). The secondary spread is quick due to hyphal growth produced from the propagules which have colonised the

root cortex (Nehl *et al.*, 1998). Primary infection still occurs throughout the secondary spread (Pattinson *et al.*, 1997) ensuring the survival of the fungi along the majority of the root zone.

The symbiosis between AMF and plants is of considerable ecological importance (Torrisi *et al.*, 1999). Symbiosis between AMF and the host plant begins when the hyphae comes in contact with the roots and forms an appressorium (Gerdemann, 1968; Harrison, 1999). The next step in the symbiotic relationship is the penetration of hyphae into the root. The hyphae can enter the root between two epidermal cells (Figure 9) or physically penetrating the cell wall of a root hair or epidermal cell (Harrison, 1999). Once the fungi have entered the host plant's root, arbuscules begin to form in the cortical cells (Gerdemann, 1968). Figure 9 illustrates the penetration of hyphae and the development of the arbuscule which is where the transfer of P to the host plant occurs (Harrison, 1999). It is well known that AMF utilises carbon from the plant. However recent work with ^{13}C nuclear magnetic resonance spectroscopy suggest that the source of carbon is glucose (Harrison, 1999; Hattingh *et al.*, 1973; Smith *et al.*, 2001).

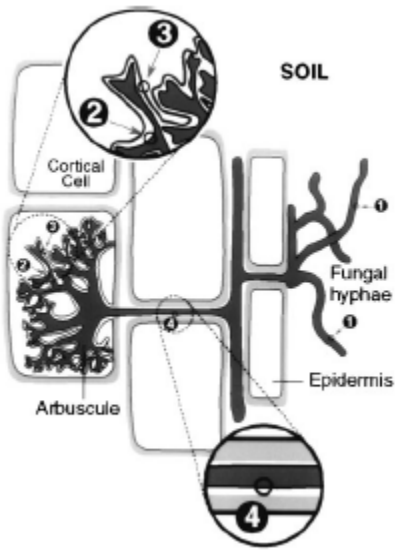

Figure 9: Infection and symbiosis between AMF and host plant. (1) P uptake by external hyphae. (2) P efflux across the arbuscule membrane. (3) P uptake by the plant across the arbuscular membrane. (2) and (4) are possible sites of glucose uptake by the fungus (Harrison, 1999).

(2) ROLE OF AMF AND EFFECT ON PLANT REPRODUCTION AND YIELD

There are many reviews illustrating the effects of AMF on the growth rates of plants and the function of symbiosis in acquiring nutrients (Abbott & Robson, 1982; Bolan, 1991; Gerdemann, 1968; Pairunan *et al.*, 1980). This is important in understanding the interactions between AMF and crop plants. However little research has been done on interactions with plant reproduction (Koide, 2000). The flowers, fruits and seeds of a commercially grown crop are generally the most important parts of the plant (Koide, 2000), relating to productivity and the economical importance. AMF may play a role in the hormonal characteristics of plant growth. AMF may increase tiller production of some grasses such as *Agropyron smithii* (Bush, 1995; Koide, 2000; Miller *et al.*, 1987).

There are many possible mechanisms of AMF by which it benefits the host plant. AMF absorbs nutrients from the soil directly into plant soils and via fungal symbiont (Smith *et al.*, 2001). Physical exploration of the soil is probably the major attribute of AMF that improves nutrient uptake (Bolan, 1991). The fact that AMF increases the host's root material permits a larger volume of soil to be accessed for nutrient exploration. Providing a 'short-circuit' to the diffusion is another mechanism of AMF that improves a plant's nutrient status (Bolan, 1991). P is generally taken up by plants by diffusion and it is said that the hyphae decreases the distance for diffusion to occur. Research has been undertaken to confirm that AMF increase absorption surfaces, where a form of available P placed 27cm from a mycorrhizal root was accumulated by the plant (Hattingh *et al.*, 1973). The absorption surface by which P can enter the plant is also increased (Bolan, 1991) due to the extensive hyphal network of AMF. Although AMF improves the uptake of P, it is proven that roots infected with AMF and those which are not obtain P from the same pool (Pairunan *et al.*, 1980).

There have also been studies on the direct and indirect mechanisms of improving nutrient availability to plants. Ectomycorrhizal fungi can directly solubilise immobile P by producing acids which break down the P into available forms (Bolan, 1991; Schachtman *et al.*, 1998). AMF may also act as a depleting agent of P in soil solution. This indirect mechanism coincides with the natural P cycle where unavailable P solubilises over time into labile forms (Bolan,

1991). This cycle may be enhanced by the presence of AMF as equilibrium of P in soil is required.

AMF plants have improved rates of photosynthesis (Auge, 2001). Plant roots which have interactions with AMF can dry out a soil relatively quickly compared to a root system absent of the fungi (Auge, 2001). In drought AMF increases growth rates and water use efficiency of host plants (Auge, 2001). Certain crop plants are dependant upon AMF than others. In the case of linseed (*Linum usitatissimum* L.), uptake of P and Zn improved when the fungi was present (Thompson, 1996).

Abutilon theophrasti is a weedy annual in the same family as cotton, *Malvaceae* (Koide, 2000). It has similar characteristics to cotton, being indeterminate in growth and the majority of the plants life cycle consists of the reproductive phase (Koide, 2000). Infection by AMF decreases the time taken to flower and it increases the proportion of flowers to fruits of *A. theophrasti*. The number of seeds per fruit is also increased by 500% at low P and only 12% at higher levels of P (Koide, 2000). This suggests that infection by AMF has a direct relationship with yield of *A. theophrasti*. Seed quality is also improved in *A. theophrasti* which benefits the next generation of the plant, ensuring survival (Koide, 2000).

(3) SURVIVAL AND LIMITATIONS OF AMF

If AMF is separated from its host or disrupted in some form, its source of carbon is lost which may decrease the concentration of the fungi in the soil (McGee *et al.*, 1997). In the Australian cotton growing areas, propagules which survive the fallow period are presumed to be spores and fungi found in root debris (McGee *et al.*, 1997). However, root density in cotton fields is low and due to quick rates of decomposition, roots are unlikely to accommodate spores over a long period (Pattinson *et al.*, 1997). In the case of soils used for the production of cotton, survival of AMF as hyphae and spores (McGee *et al.*, 1997) is important for future infection. Many species of AMF have diverse survival periods. Hyphae of *A. laevis* started to decline after 11 weeks in dry soil with no host present (Jasper *et al.*, 1993) whereas hyphae of *S. calospora* survives for a longer period in the same conditions (Jasper *et al.*, 1993).

High plant densities decrease the colonisation by AMF. As plant numbers increase the root density also increases, this impedes the AMF to root ratio. Vegetative and reproductive responses to AMF tend to decline in *A. theophrasti* when plant density is increased (Koide, 2000).

Colonisation of host roots occurs much faster in undisturbed soil than cultivated soil, and infection occurred after 4 days and up to 15 days respectively (McGee *et al.*, 1997). The percentage of roots infected for the two soil types were 45% and 30% respectively after 42 days (Figure 10) (McGee *et al.*, 1997). The presence of high fungal growth coincided with nutrient availability, especially P which might increase yield and reduce fertiliser costs.

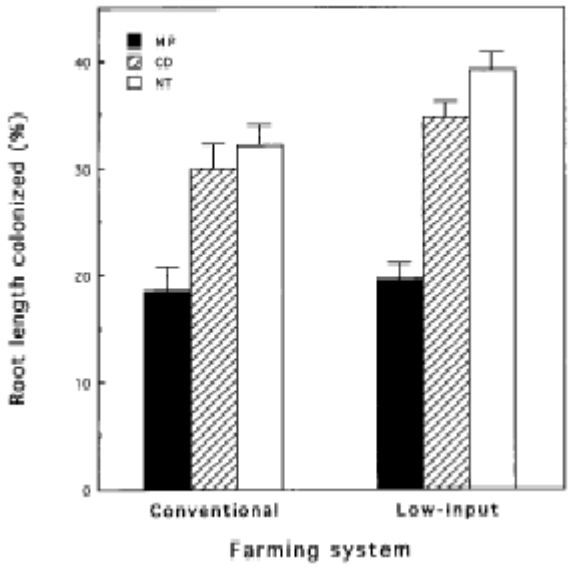

Figure10: Colonisation of roots of *Paspalum notatum* in soils manipulated in different ways. MP- Moldboard Plough, CD- Chisel-Disk, NT- No till (Galvez *et al.*, 2001)

Periodic wetting and drying could also affect the presence of AMF. Wetting of undisturbed and disturbed soils used for cotton production severely alters the rate and amount of infection of the fungi (Pattinson *et al.*, 1997).

VII. CONCLUSION

The removal of nutrients from a cropping system is inevitable as the profitable portion of the crop is generally high in essential nutrients. Intensive agriculture frequently relies on inputs such as fertilisers to amend the problem associated with nutrient deficiency. As cotton production enters marginal growing areas of Australia, such as Kununurra, determining the optimum amount of P fertiliser which will produce profitable yields will be an important aspect of cotton production. Soil is considered P deficient when Colwell P is less than 6mg/kg (Constable *et al.*, 2001; Dorahy *et al.*, 2004). It is well known that P is relatively immobile in the soil and difficult for the plant to acquire (Constable *et al.*, 2001; Koide *et al.*, 1999). Observing yield components will be a useful tool for collecting quantitative data to substantiate where yield derives from. Establishing the source of a high yields, such as number of reproductive parts or number of seeds per unit area will enable breeders to emphasise on particular traits to develop superior plants.

Many studies have been performed concerning the interaction of AMF and P uptake of plants regarding plant growth and nutrient transfer (Gerdemann, 1968; Harrison, 1999). However, there is a need to understand the relationship between AMF and the reproductive features of plants, particularly cotton. Reproductive components including flowers and seeds usually determine yield and ultimately the productivity and profit of a commercially grown crop (Koide, 2000). It is well known that AMF obtain carbon from the host plant. More information on the responses of reproductive and vegetative parts are needed to understand the effects on yield (Koide, 2000). P fertiliser is often applied to agricultural soils and has been suggested to suppress the growth and performance of AMF (Miller *et al.*, 1995). More information on how plants react in different situations regarding P and AMF on marginal soils will benefit producers in these regions.

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PAPER 2 - RESEARCH PAPER

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Introduction

Australian soils are characteristically low in phosphorus (P) in their native state (Incitec Pivot 2003). Agriculture can further deplete soil fertility if not managed in a sustainable manner. Most of the P in soils is associated with organic matter, usually between 20 and 80% is present in organic forms (Incitec Pivot 2003). P is one of the most important macronutrients essential for plant growth. After nitrogen, P is the most limiting element for plant growth (Vance 2001). It is present in nucleic acids, phospholipids and ATP (Schachtman, Reid *et al.* 1998). P is involved in two important transformations of energy in plants, being adenosine diphosphate (ADP) and adenosine triphosphate (ATP) (Penfold 2000). P also plays a role in regulating metabolic pathways and certain enzyme reactions (Theodorou and Plaxton 1993).

The amount of P found in plants varies from 0.05 to 0.30% of dry weight (Vance 2001), depending on species and conditions. P is an important element in late season crop nutrition. P is associated with premature senescence where waterlogging or unfavourable growing conditions limit P uptake, reduce P leaf concentration and result in reduced photosynthesis and metabolism causing senescence (Constable, Deutscher *et al.* 2001).

P is most available to plants growing in a soil pH range of 6.5 to 7.5. The soil pH influences the length of time P fertilisers can remain in plant available forms (Dorahy, Rochester *et al.* 2004). P applied as fertiliser is relatively immobile in soil and often requires soil incorporation to move any distance. Plants require a large amount of P, especially early in the growing season as it plays an important role in stimulating root development. Unlike soil, P is readily moved within the plant from old to young tissue in P deficient plants. In plants with adequate P supply, the majority of the P absorbed is transported in the xylem to the younger leaves (Schachtman, Reid *et al.* 1998). The best responses to P fertiliser are obtained from early application (IncitecPivot 2003), usually prior to planting in cotton and other annual crops.

The soils of the Ord River Irrigation area (ORIA) are naturally low in available P. The production of cotton requires the application of fertilisers to provide a sustainable cropping system. The critical limits of P in cotton range from 5 to 12 mg/kg (Hibberd *et al.* 1990) with

availability declining with increasing soil pH. There has been some research on P requirements of wet season cotton grown in the ORIA (Thomson and Basinki 1962). The authors suggested 22 kg/ha of P applied as superphosphate to be a suitable rate for the production of a profitable crop. This research was performed on obsolete cultivars and used traditional cultural practices which vary considerably to today's methods due to technology advancements. The tropical climate, dry season cotton and soils with low available P presents a situation where traditional fertiliser requirements could be challenged because of unknown responses of modern transgenic cultivars. Research on cotton yield response to P has been studied at some depth (Dorahy *et al.* 2004; Hibberd *et al.* 1990). However, little work has been done on the effect of applied P on cotton yield components.

Arbuscular mycorrhiza fungi (AMF) are symbiotic fungi that feed on carbohydrates of many agronomic crops which in turn increase plants root mass. AMF belong to the Zygomycota family consisting of one order Glomales, and 6 genera where 149 species have been classified (Harrison 1999). AMF increase the surface area of the roots which allows the plant to have greater access to nutrients such as P. P is generally taken up by plants by diffusion and the hyphae decreases the distance for diffusion to occur. AMF can also transfer moisture to the plant (Auge 2001; Gerdemann 1968). The relationship between AMF and many crop species have been described (Koide 2000; McGonigle *et al.* 2003). However, little work has been performed on the role of AMF in relation to yield of cotton.

The objective of the field experiments was to test the hypothesis that the application of P will increase yields and yield components of cotton grown in virgin soil in the ORIA. The objective of the glasshouse experiment was to test the hypothesis that the application of P and/or addition of AMF will increase cotton phosphorus uptake and dry weight.

Materials and methods

Field experiment

Experiments were conducted at the Frank Wise Institute of Tropical Agriculture, Kununurra, WA, Australia in the 2002 and 2003 during the dry season. The virgin soil on which the field experiments were conducted had been progressively cleared of native vegetation such as

Sorghum plumosum, *S. australiense*, *Themeda triandra* and *Astrebla squarrosa*. The soil is known locally as "Cununurra clay" and is uniform dark brown medium to heavy clay with shrink-swell properties. Soil analysis was conducted on the fields during the 2001 dry season and analysed by at the Waite Institute, Adelaide. The soil tests indicated 3 mg/kg of available Colwell P and 31 mg/kg of total P was present. The total plant available P was calculated by the Colwell bicarbonate extraction method (Colwell 1963). The soil pH (1:5 $CaCl_2$) was 6.91 and tended to increase with depth (Duggan and Ryan 2004). Apart from manganese all other nutrients were comparable to industry standards.

Prior to sowing in 2002 and 2003, five rates of phosphorus (0, 40, 80, 120 and 160 kg/ha) were applied as double superphosphate (17.5% P) 20 cm deep and 2 cm adjacent to the proposed plant line. The experiment used a randomised complete block design with four replicates of each phosphorus rate. The crops were sown into dry soil on the 28th of April 2002 and on the 28th of March 2003. The cotton cultivar (*Gossypium hirsutum*) sown in 2002 was Sicot 289i (Ingard®) while in 2003 the cultivar was Sicot 289B (Bollgard II®). At harvest plants were sampled from $1m^2$ quadrants in each replicate. Plant mapping was carried out on the samples. The bolls were then removed from the plants and ginned. The lint and seed from each boll was weighed for analysis. AMF was measured in the field experiment. AMF colonisation in 2002 was low (<11% of root length) 17 days after planting while in 2003 it increased slightly (25% of root length) (Duggan and Ryan 2004). AMF did not play a major role in crop growth at time of sampling.

Cultural practices

The land was laser levelled in 1996 and 2001. The cotton from Kununurra was planted into virgin ground in 2002 and a new area again in 2003. Prior to sowing the land was maintained with use of herbicides such as 2,4-D amine, paraquat and glyphosate to control weeds. Apart from P fertiliser, the cotton plants were also fertilised with 200 kg/ha N as urea, 51 kg/ha S and 40 kg/ha Zn as $ZnSO_4$. A further application of $ZnSO_4$ heptahydrate was applied at 1 kg/ha as a foliar fertiliser. This was applied to the plants as they lacked vigour and had a low Zn concentration which was supported by typical Zn deficiency symptoms (short plants with interveinal chlorosis and leaf cupping).

The beds were watered up 2 days after sowing and 9 plants/m were established on beds 1 m apart. Weed control was implemented by incorporating pre-emergent herbicide and removal by hand before the cotton had reached first flower. The two cultivars used contained the Bt protein which provided control against *Helicoverpa armigera* and *H. punctigera.* No significant rainfall events occurred during the growing season. Irrigation was scheduled based on pan evaporation.

Plant mapping

Plant mapping was carried out to provide data that could be statistically analysed for varying responses to P fertiliser. Plant mapping was conducted on ten plants per plot prior to harvesting.

The measurements taken include:

- Plant number
- Plant height
- Total node number
- Total fruiting nodes
- Total vegetative nodes
- Total bolls
- Retained bolls

The total nodes include nodes that have been aborted. Up to the first 5 to 7 nodes are aborted leaving a scar. The coleoptile scars are not counted as nodes. Fruiting nodes are the fruiting branches that have the ability to produce fruit. Vegetative nodes commonly occur low on the plant. They are branches that will not produce fruit. However, fruiting nodes can grow off a vegetative branch and produce fruit. Vegetative nodes may also occur when the fruiting nodes have been damaged or tipped out. Tipping out of a plant could be due to poor nutrient or water status or mechanical damage by insects or other pests. The total number of bolls and their position on the plant was also recorded. Squares or young bolls may have been shed early due to water or nutrient stress or by insect damage so it is important to count all the fruiting sites to determine the rate of retention. From the data collected by plant mapping the cotton plant, analysis was performed to determine any significant responses to P fertiliser.

Yield components

The seed cotton from Kununurra was removed and transported to the Australian Cotton Research Institute in Narrabri for ginning and the University of Sydney, Sydney for further analysis. Seed cotton is the unprocessed seed with lint still attached to it, after picking. The following measurements were made in order to calculate cotton yield components.

- Seed cotton weight (g)
- Seed weight (g)
- Seed number
- Handpicked lint kg/ha
- Machine picked lint kg/ha

The seed cotton was weighed before being ginned. The small saw gin was used for the experimental work and operates by separating the lint from the seed and removing any trash in the sample. A ratio of lint to seed weight (lint percentage) per sample was attained by weighing the seeds after ginning. The number of seeds was also counted. The weight of the lint was attained by subtracting the mass of the seeds from the original seed cotton weight. The plots in Kununurra were also mechanically harvested by a single row picker to obtain independent yield data.

(a)

(b)

Fig. 1. (a) Seed cotton being weighed before further processing (b) lint and seed are important yield components.

The yield component data can be described using the following equation to help explain the response of yield to P fertiliser.

Equation 1. Yield = bolls/unit area x lint/boll (Kerr 1966)

This equation can be further broken down to:

Equation 2. Yield = fruiting sites/m^2 x boll retention x seeds/boll x lint/seed (Worley *et al.* 1974)

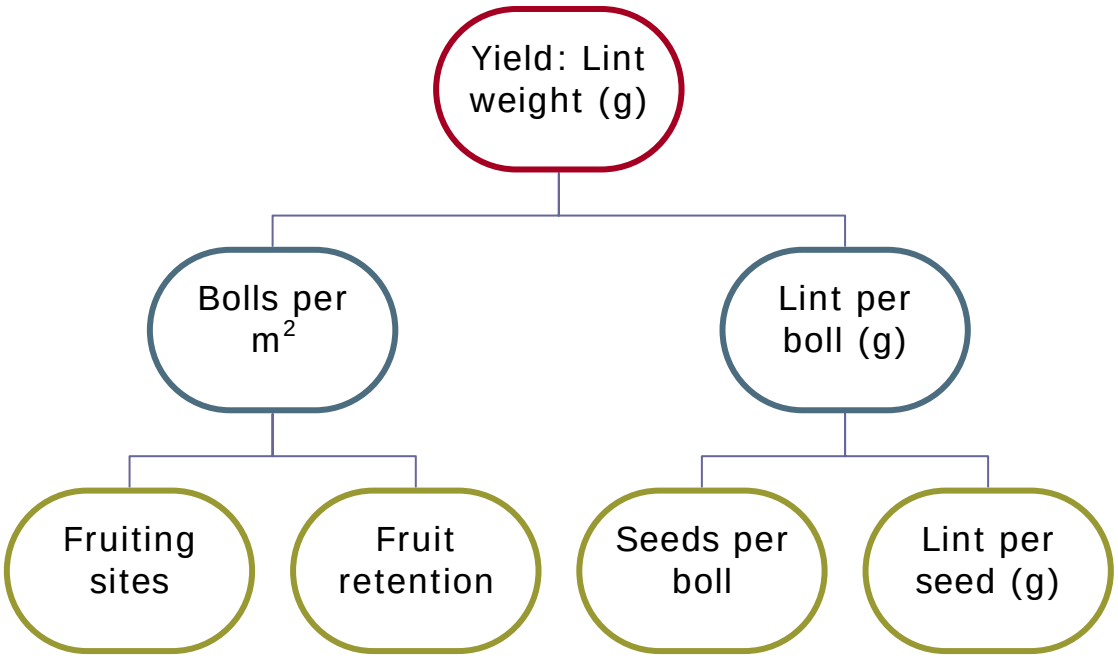


Fig. 2. Cotton yield can be broken down into yield components.

Glasshouse experiment

The glasshouse experiment was carried out in the Darlington glasshouse at the University of Sydney. To simulate climatic conditions of cotton production, the glasshouse was maintained at a day/night temperature of 30/20 ^{o}C. Sodium lamps were installed and set to maintain a photoperiod of 16 h light each day. An automatic watering system was set up to irrigate 20 mL per pot per day. Soil was collected from "Koarlo", a property known for having phosphorus deficient soils, east of Goondiwindi in south-eastern Queensland. The soil was transported to Sydney and gamma irradiated with a dose of 75 kGy by Steritech Pty Ltd to kill all soil microbes. The nutritional status of the soil was assessed to determine total P and available Colwell P. The soil had 10 mg/kg of available P and a pH (1:5 $CaCl_2$) of 7.6.

The cotton seeds were germinated in autoclaved sand in the glasshouse. If the seed coats remained after emergence they were carefully removed, releasing the cotyledons. The seedlings were transplanted when two true leaves appeared, approximately one week after planting. The seedlings were transplanted on the 6th July 2006 into pots containing a mixture of the "Koarlo" soil (60%) and sterilised course sand (0 P) (40%) to ensure adequate drainage. The pots were relatively deep to accommodate the large cotton tap roots. Arbuscular mycorrhizal fungi (AMF) was added as a soil mixture of colonised roots to the respective treatments requiring inoculation. The soil and root mixture was separated into 1 cm segments and placed beneath the seedlings prior to transplanting. This would ensure that the seedling roots would penetrate through the inoculum and form a symbiotic relationship. The presence of AMF was verified by collecting fine roots of all treatments. The fine roots were placed in 10% KOH and heated for 3 minutes until the roots appeared transparent. The roots were rinsed with 1% HCl as the stain only works in acidic conditions. The roots were placed in 0.05% trypan blue in lactoglycerol. After staining the roots were placed on slides and examined under a compound microscope to determine proportional colonisation of AMF (McGee *et al.* 1997). At the end of the experiment on 28th of October 2006, AMF was confirmed by the proportional colonisation method. The glasshouse experiment consisted of a factorial experiment with four treatments and eight replicates in a completely randomised block design (32 pots and 96 plants).

Table 1. Glasshouse treatments

	Treatment
1	- P and -AMF (control
2	+ P and -AMF
3	- P and + AMF
4	+ P and + AMF

The treatments were fertilised with a modified Hoagland's solution (see table 3). The treatments without P were fertilised with the same solution minus KH_2PO_4.

Table 2. The standard nutrient mix for the glasshouse plants

Nutrient	**Standard Mix** mg of nutrient per kg of dry soil	Nutrient solution	Solution conc.	mL of solution added per kg of dry soil
N	400	NH_4NO_3	2M	4.7
Ca	120	$Ca(NO_3)_2$	1M	3.8
K	250	KNO_3	1M	3.0
P	80	KH_2PO_4	1M	2.6
Mg	72	$MgSO_4$	1M	3.0
S, Fe, B, Mn, Zn, Cu, Mo, Co		Librel® at 2mL per kg of soil		

The cotton in the glasshouse was checked regularly for damage by pests. The control of aphids and two spotted mites (*Tetranychus urticae*) was achieved by taking an integrated pest management approach. Confidor®, a systemic insecticide was used to control aphids and predatory mites (*Phytoseiulus persimilis*) were released to control two spotted mites.

The cotton from the glasshouse was harvested on the 16th of October 2006 and separated into stems and leaves. The samples were dehydrated at 70°C in a forced-air dryer for 6 days and the youngest mature leaf (YML) and stems were digested using the molybdovandate method (Polyzopoulos *et al.* 1982) to measure P concentration in plant material The digested samples were measured using a spectrophotometer at wavelength 460 nm and set against a standard to determine the amount of P present. The concentration of the sub-sample was multiplied by the total dry weight of each plant part (stems and leaves) to determine overall P uptake.

Data analysis

Field experiment

The statistical analysis was performed using the statistical program Genstat v8. Probability plots and residual analyses were conducted on the data to ensure normality. Standard curves were fitted to the data sets to develop a model of the response to P treatment. The models were based on a growth response curve and fitted using an exponential (asymptotic) model (equation 3).

Equation 3. $\boldsymbol{Y = A + B(R^x)}$

The r^2 and F-test *P* values were calculated for each model to estimate its goodness of fit and statistical significance. If the data was not normally distributed, data was transformed using the natural log function to ensure the variance was constant. Yield components were plotted against lint yield to determine the contribution of each component to yield.

Glasshouse experiment

Analysis of variance was performed on the data collected from the glasshouse cotton plants using Genstat v8. If the data was not normally distributed, data was transformed using the natural log function to ensure the variance was constant.

Results

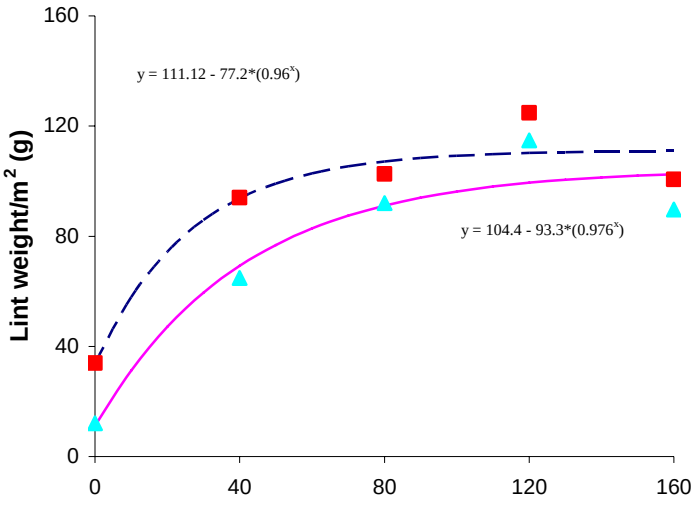
Field experiment

There was a significant yield response to P fertiliser to approximately 80 kg/ha P fertiliser in both 2002 and 2003 (Fig. 3*a*). The yield increased from 40 g/m^2 at 0 P to approximately 100 g/m^2 at 80 kg/ha P in 2002 and from 12 g/m^2 to 85 g/m^2 in 2003. The amount of P fertiliser applied both in 2002 and 2003 gave differential responses to the yield components. The most distinguishing difference was observed between the 0 P treatments and the rest of the P treatments.

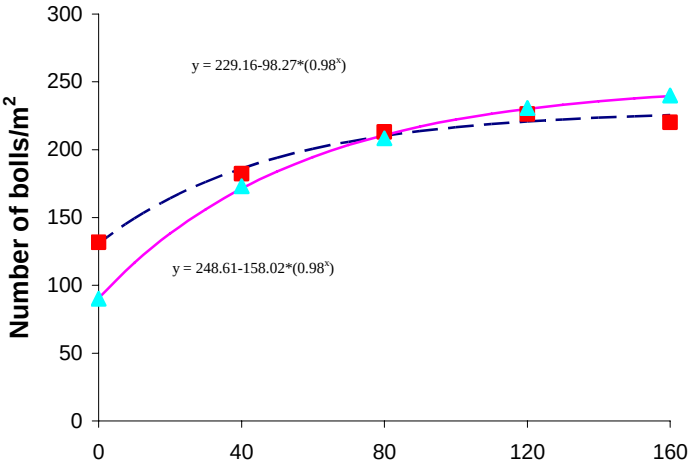
Bolls/m^2

The application of P fertiliser did not affect the number of fruiting nodes or fruiting sites. The yield component contributing most to the yield was the number of bolls per m^2 (Fig. 3*b*). The number of bolls/m^2 contributed 94% and 83% to the increase in total lint/m^2 (yield) due to P fertiliser application in 2002 and 2003, respectively (Fig. 4*a, b*). Boll numbers per m^2 can be broken down further into fruiting sites and boll retention. As percentage boll retention increases, the number of bolls/m^2 increase (Fig. 5*a, b*). Hence, percentage boll retention was the most important factor contributing to the increase in bolls per m^2 (Fig. 3*c*) rather than the number of fruiting sites.

(a)



(b)



Double superphosphate kg/ha

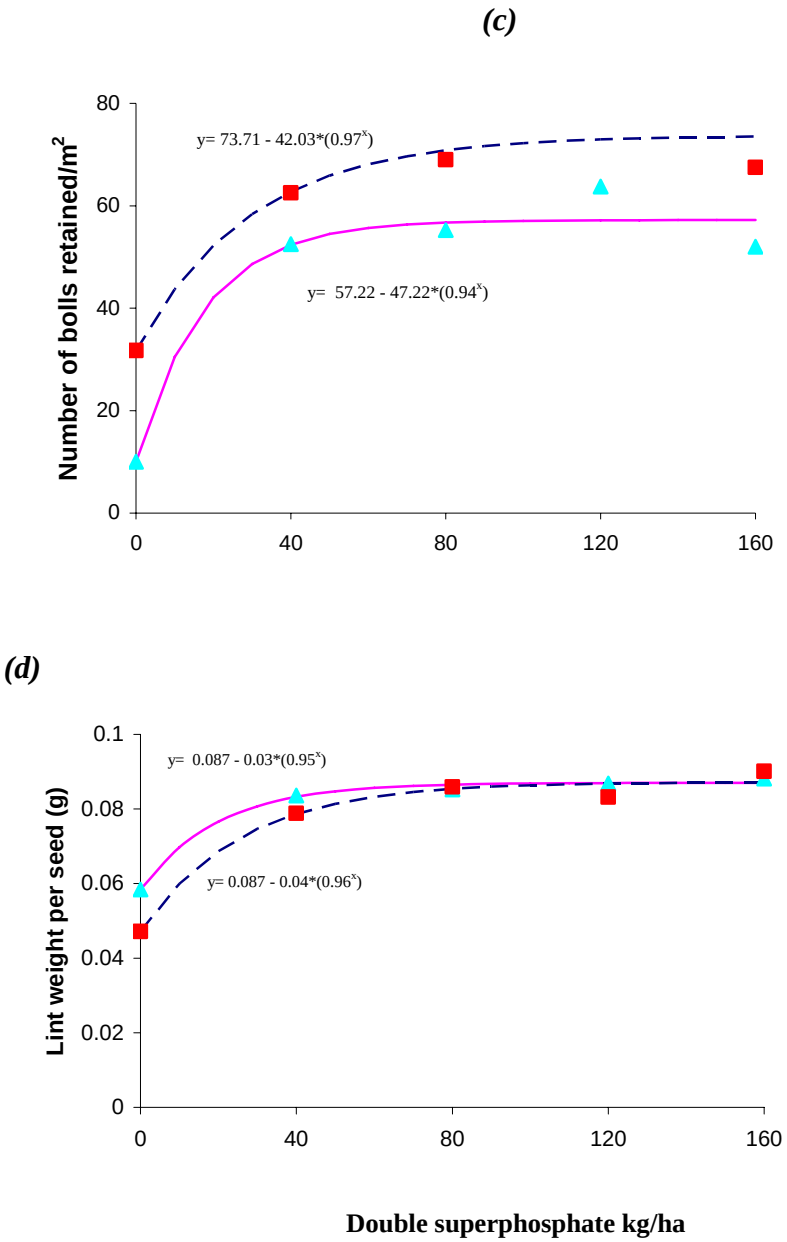


Fig. 3. ***(a)*** Response of cotton lint/m^2 (yield) (2002 r^2 = 0.96, 2003 r^2 = 0.98, P < 0.01), ***(b)*** number of bolls/m^2 (2002 r^2 = 0.98, 2003 r^2 = 0.98, P < 0.01), ***(c)*** percentage bolls retained per plant/ m^2 (2002 r^2 = 0.97, 2003 r^2 = 0.94, P < 0.01) and ***(d)*** lint weight/seed (g) (2002 r^2 = 0.96, 2003 r^2 = 0.95, P < 0.01) to application of P fertiliser. The dashed line is 2002 fitted line, solid line is 2003 fitted line, the solid squares are means of 2002 data and the solid triangles are means of 2003 data.

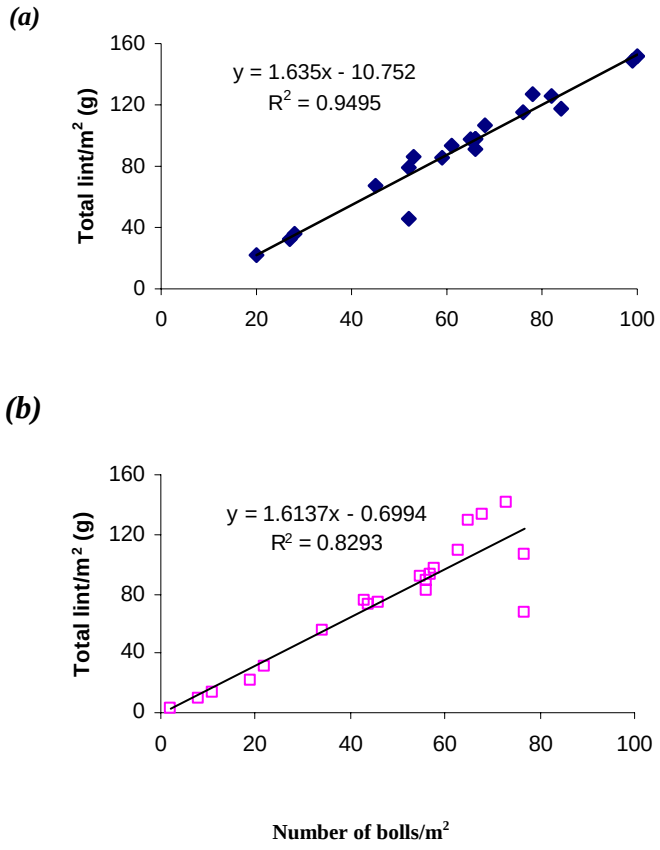
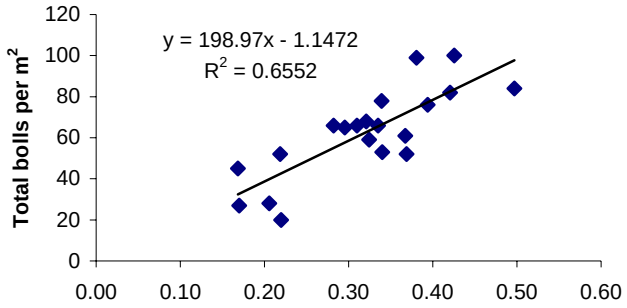


Fig. 4. Contribution of bolls/m^2 to the increase in total lint/m^2 (yield) (g) due to P fertiliser application for ***(a)*** 2002 ***(b)*** 2003.

(a)



(b)

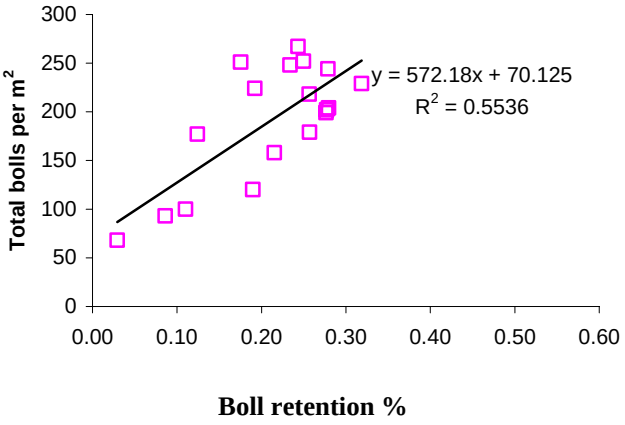


Fig. 5. Contribution of percentage boll retention to the increase in total bolls per m^2 due to P fertiliser application in ***(a)*** 2002 and ***(b)*** 2003.

Lint/boll

Average weight (g) of lint per boll contributed 54% and 60% to the increase in total lint weight/m^2 due to P fertiliser application in 2002 and 2003, respectively (Fig. 6*a*, *b*). Lint weight/boll (g) can be further broken down to lint/seed (g) and seeds/boll. Lint weight/seed contributed 64 and 65% to the increase in weight of lint/boll (g) due to P fertiliser application in 2002 and 2003, respectively (Fig. 7*a*, *b*). Seed/boll did not have any relationship (P>0.05) to lint/boll (Fig 8a, b).

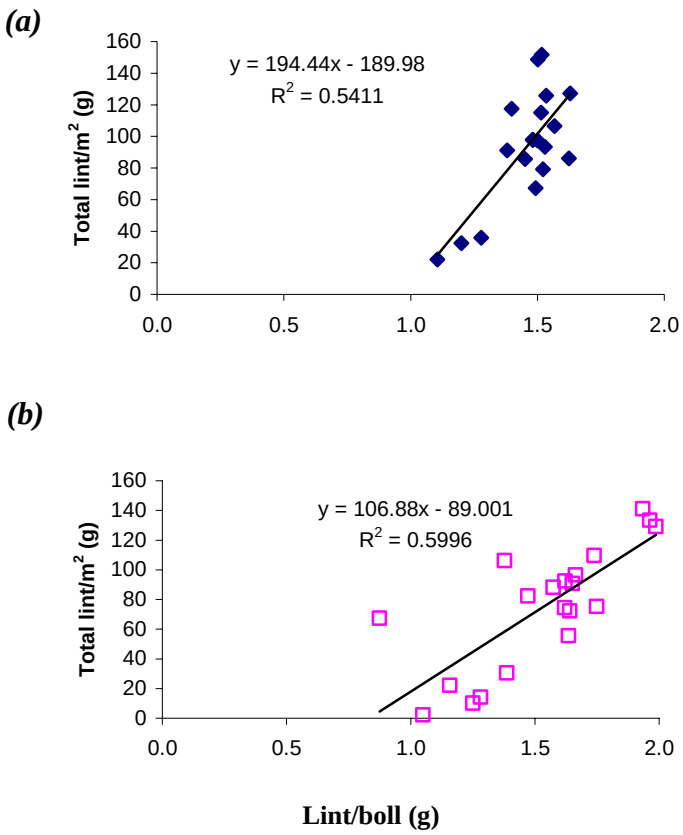


Fig. 6. Contribution of lint/boll (g) to the increase in total lint/m^2 (yield) due to P fertiliser application in ***(a)*** 2002 ***(b)*** 2003.

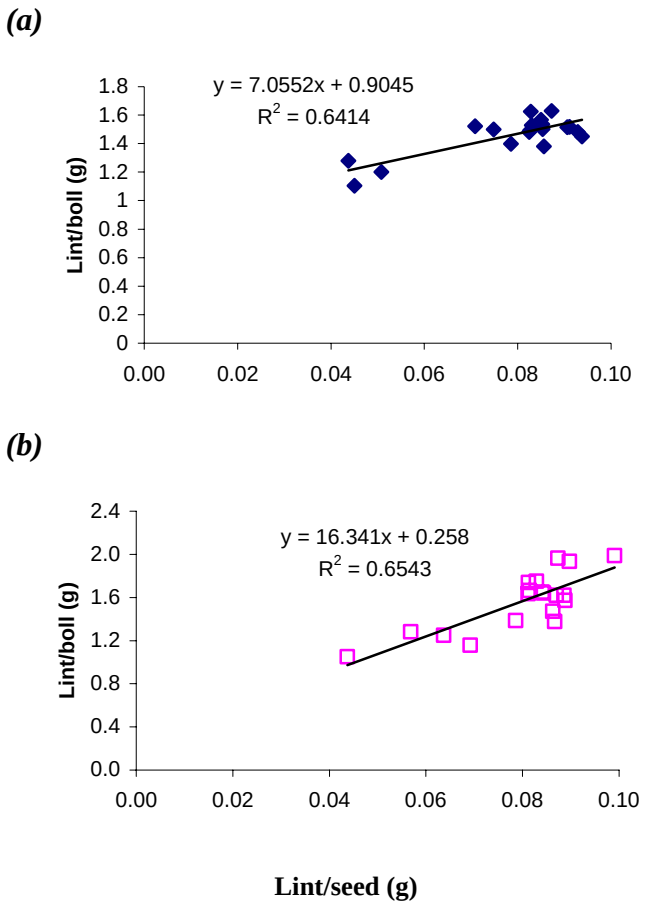


Fig. 7. Contribution of lint/seed (g) to the increase in yield lint/boll (g) due to P fertiliser application in ***(a)*** 2002 ***(b)*** 2003.

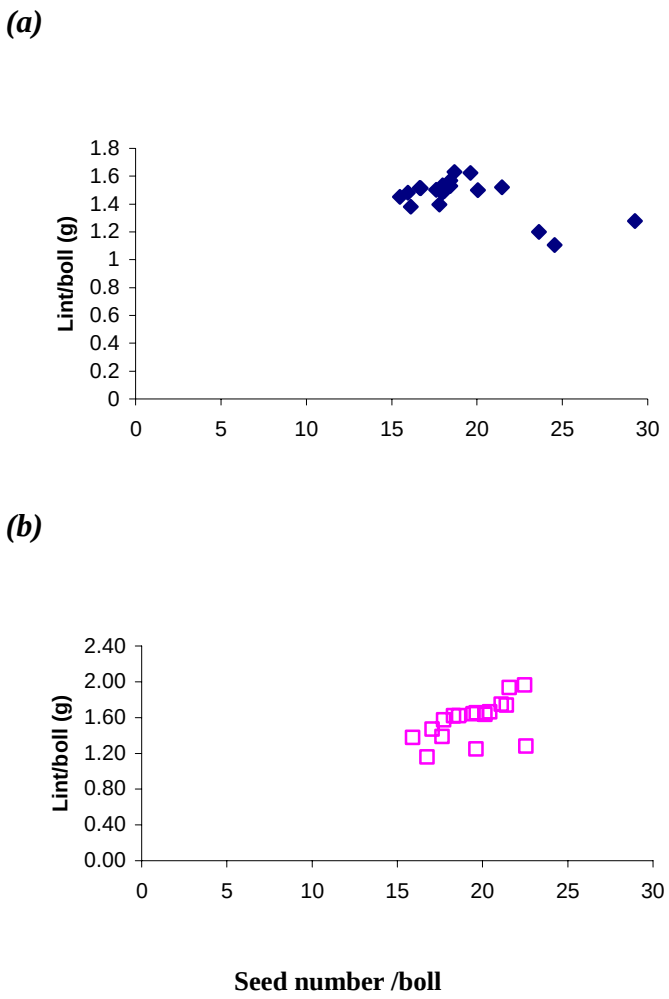


Fig. 8. No obvious relationship between seed number/boll and lint/boll (g) in ***(a)*** 2002 and ***(b)*** 2003

The increase in lint yield is primarily attributed to bolls/m^2 and to a lesser extent by lint/boll. Percentage fruit retention contributed most to the increase in bolls/m^2 and lint/seed contributes most to the increase in lint/boll. The number of fruiting sites and seeds/boll do not contribute much to the increase bolls/m^2 and lint/boll, respectively.

Glasshouse experiment

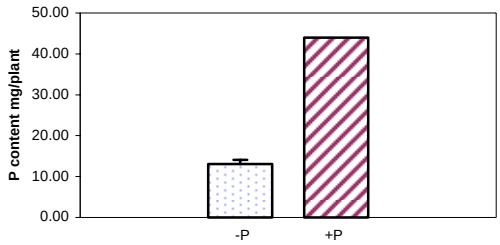
There was a difference (P<0.01) in all variables measured for P fertiliser main effects (Table 3) There were no AMF main effects and no interaction between P and AMF (P>0.05). The plants that received P fertiliser had higher (P<0.01) P uptake than the – P treatments (Fig. 10*a*). The + P treatments also showed higher stem, leaf and total dry weights (Fig.10*b, c, d*) than – P treatments. Plants with sufficient P had a P concentration of ~3 300 mg/kg while -P plants had lower (P<0.01) P concentrations (~ 1 500 mg/kg).

Table 3. F-test P values of variables analysed to determine cotton growth and P uptake in the glasshouse experiment. ** Significant at P<0.01, n.s. - not significant at P=0.05)

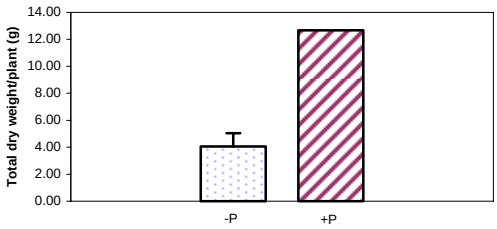
Variable	P	AMF	P x AMF Interaction
Total P/plant	**	n.s.	n.s.
Total dry weight	**	n.s.	n.s.
Stem dry weight	**	n.s.	n.s.
Leaf dry weight	**	n.s.	n.s.

Fig. 9. –P deficient cotton grown in glasshouse exhibit symptoms of P deficiency. Stunting and dark green leaves are an obvious sign.

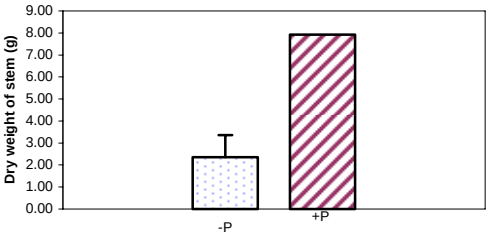
(a)



(b)



(c)



(d)

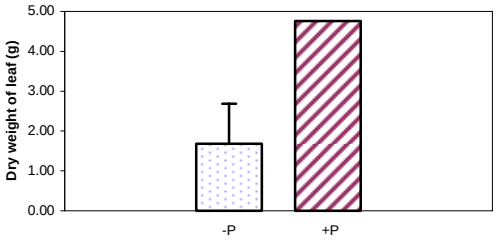


Fig. 10. ***(a)*** P uptake per plant (mg) ***(b)*** total dry weight (g) ***(c)*** stem dry weights (g) per plant and ***(d)*** leaf dry weight (g) per plant for – P and + P treatments. Vertical bars represent l.s.d. at *P*=0.05.

Discussion

Field experiment

The role of fertilisers is to provide specific nutrients, in this case P, in order to obtain a yield of 90% or more of its potential yield (Morel and Fardeau 1990). A rate of 80 kg/ha of P applied as double superphosphate (17.5% P) was required to optimise yield for dry season cotton production in the ORIA in 2 virgin soils (Fig. 2*a*). The rate of 80 kg/ha used in this experiment was higher than the 22 kg/ha of P as superphosphate suggested by (Thomson and Basinki (1962) for ORIA.

Yield components

Studying the yield components with respect to P fertiliser application rates will determine which yield component has the greatest contribution to the increase in yield in response to P. The number of bolls/m^2 was suggested to be the most important contributor to lint yield, followed by seeds/boll and lint/seed (Worley *et al.* 1974). The results in this study supported this hypothesis as bolls/m^2 contributed most (r^2 = 83-95%) to the increase in lint yield due to P fertiliser followed by lint/boll (r^2 = 54-60%). Fruiting sites per plant did not significantly contribute to the increase in cotton yield. Percentage boll retention has an impact on bolls/m^2 and hence, contributes to the increase in total lint weight/m^2 (Fig. 2*c*, 4*a & b*). Many studies have shown that lint yield was influenced by P (Dorahy *et al.* 2004; Hibberd *et al.* 1990; Howard *et al.* 2001). However, boll retention due to P nutrition has not been studied in detail. The retention of bolls contributed (r^2 = 55-65%) to yield. Boll retention was relatively higher in 2002 (Fig. 4*a, b*) than in 2003. This may be due to external stresses such as water or heat or mechanical damage, which lead to a higher rate of fruit shedding. Bolls are also shed because there are not enough resources to carry all of the bolls through to maturity(Constable *et al.* 2001).

Lint/boll (boll size) was increased due to the increase of lint/seed (r^2 = 64-65%) with P fertiliser application. This is consistent with previous work showing that boll size and lint percentage were important components that determine cotton yield (Iqbal *et al.* 2003; Worley *et al.* 1974). P deficiency often delays fruiting and maturity (Constable *et al.* 2001). In this study, the number and size of the fruit was reduced in the P deficient plants which is consistent with Constable *et*

al. (2001)'s work. Seeds per boll had no significant influence on total lint yield (Fig. 7*a, b*). If a cotton plant depends on a high number of seeds per boll to produce a profitable yield, the plant has to fix a large amount of carbon to achieve such a result. In terms of energy requirement, the cotton plant must fix nearly twice as much carbon to produce a kilogram of seed compared to a kilogram of lint (West and Todd 1956). This is because cotton seed contains approximately 20% triglyceride, or oil (Lewis *et al.* 2000). By selecting for high seed number for yield production, cotton yields can become more variable and less reliable(Lewis *et al.* 2000).

Glasshouse experiment

P concentration

Critical levels of P in the plant depend on the plant part and the time of sampling. Usually, the youngest mature leaf is sampled. Levels of adequacy for P in the youngest mature leaf at early square stage is 3 100 mg/kg (Constable *et al.* 2001). The cotton plants that received P fertiliser in this study (Fig. 2*a*) were within this range (~3 300 mg/kg). The plants which did not receive P fertiliser contained a much lower P concentration and content. Visual deficiency symptoms were noticeable, such as stunting, dark green leaves and purple discolouration of veins and delayed development (see Fig. 9).

P and AMF

P fertiliser is often applied to agricultural soils and has been suggested to suppress the growth and performance of AMF (Miller *et al.* 1995). This glasshouse study suggested that AMF has did not affect on the growth and P uptake of cotton plants. This is inconsistent with other studies which have suggested that AMF has a direct relationship with P uptake (Duggan and Ryan 2004; Gerdemann 1968; Koide 2000). *Abutilon theophrasti* is a weedy annual in the same family as cotton, *Malvaceae*. It has similar characteristics to cotton, being indeterminate in growth and the majority of the plants life cycle consists of the reproductive phase (Koide 2000). Infection by AMF decreased the time taken to flower and increased the proportion of flowers to fruits of *A. theophrasti*. The number of seeds per fruit was also increased by 500% at low P and only 12% at higher levels of P (Koide 2000). This suggests that infection by AMF has a direct relationship with yield of *A. theophrasti.* Seed quality is also improved in *A. theophrasti* which benefits the

next generation of the plant, ensuring survival (Koide 2000). AMF does not survive well in extended phases of waterlogging (Gerdemann 1968). Watering everyday may have caused some waterlogging and reduced beneficial effects of AMF. AMF was re-isolated in all AMF treatments at the end of the experiment but the percentage of root colonisation was not determined. A poor colonisation rate may have contributed to the lack of any beneficial effects by AMF.

Future work

Further work involving AMF and any interactions with yield components could be worthwhile. Only dry weights and P uptake was looked at in this study. A further look at the effect of AMF and P on yield and yield components may provide interesting data. Other work could involve looking at other essential nutrients (eg., N, K, Ca, Mg, Zn) and their influence on yield and yield components.

Conclusion

The soils of the Ord River Irrigation area (ORIA) are extremely old and naturally low in available P. The production of cotton in the area requires the application of fertilisers to provide a profitable yield. The P requirement for cotton has been studied extensively with critical limits of P reported to range from 5 to 12 mg/kg with P availability declining as soil pH increases. An optimum of 80 kg/ha of double superphosphate (17.5% P) was required on virgin soil to meet the nutrition requirements of a dry season cotton crop grown in the ORIA. Breaking down the yield into yield components helped to provide an understanding of which components contributed most to the increase in yield. The increase in lint yield is primarily attributed to bolls/m^2 and to a lesser extent by lint/boll. Percentage fruit retention contributed most to the increase in bolls/m^2 and lint/seed contributed most to the increase in lint/boll. The number of fruiting sites and seeds/boll do not contribute much to the increase bolls/m^2 and lint/boll, respectively. P was the most significant treatment in the glasshouse study. Plants which had P applied had higher P uptake and dry weights. AMF did not contribute to growth or P uptake in the glasshouse study.

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Appendix

Yield component data

Plot No.	P Level (kg/ha)	Rep	seed cotton wt (g)	seed wt (g)	lint wt (g)	Fruiting sites	Total bolls	lint per boll (g)	seeds per boll	lint per seed (g)
9	0	1	92.7	56.9	35.8	136	28	1.28	29.25	0.043712
10	0	2	58.3	36.2	22.1	91	20	1.11	24.55	0.04501
6	0	3	82.3	49.9	32.4	159	27	1.20	23.62963	0.050784
20	0	4	115.9	70.3	45.6	141	52	0.88	17.88462	0.049032
8	40	1	187.4	101.3	86.1	156	53	1.62	19.62264	0.082788
18	40	2	177.6	98.5	79.1	238	52	1.52	21.46154	0.070878
3	40	3	254.1	136.7	117.4	169	84	1.40	17.79762	0.078528
5	40	4	198.1	104.8	93.3	166	61	1.53	18.42623	0.083007
1	80	1	238.1	123	115.1	193	76	1.51	16.71053	0.09063
15	80	2	192.8	101.7	91.1	213	66	1.38	16.12121	0.08562
19	80	3	209.3	111.5	97.8	234	66	1.48	17.9697	0.082462
13	80	4	221.1	114.5	106.6	212	68	1.57	18.44118	0.085008
17	120	1	208.5	111	97.5	220	65	1.50	20.04615	0.074827
2	120	2	318.5	169.8	148.7	260	99	1.50	17.59596	0.085362
11	120	3	265.3	139.5	125.8	195	82	1.53	17.9878	0.085288
12	120	4	263.6	136.5	127.1	230	78	1.63	18.67949	0.087234
16	160	1	143.2	76	67.2	267	45	1.49	18.06667	0.082657
7	160	2	203.8	106	97.8	197	66	1.48	15.95455	0.092877
14	160	3	315.9	164.2	151.7	235	100	1.52	16.65	0.091111
4	160	4	176.5	90.9	85.6	182	59	1.45	15.47458	0.093757
32	0	1	36.2	22.1	14.1	100	11	1.3	22.54545	0.056855
31	0	2	24.9	14.9	10.0	93	8	1.3	19.625	0.063694
35	0	3	52.8	30.8	22.0	100	19	1.2	16.73684	0.069182
21	0	4	5.7	3.6	2.1	68	2	1.1	24	0.04375
33	40	1	233.0	126.9	106.1	191	77	1.4	15.8961	0.086683
23	40	2	124.8	69.2	55.6	158	34	1.6	20.14706	0.081168
38	40	3	145.0	77.7	67.3	166	77	0.9	19.9	0.087859
36	40	4	67.4	36.9	30.5	177	22	1.4	17.63636	0.078608
40	80	1	200.7	108.3	92.4	204	57	1.6	18.29825	0.088591
26	80	2	164.6	90.1	74.5	179	46	1.6	18.6087	0.087033
22	80	3	194.9	104.1	90.8	199	55	1.7	19.63636	0.084074
28	80	4	243.9	134.4	109.5	252	63	1.7	21.39683	0.081231
25	120	1	289.7	156.2	133.5	244	68	2.0	22.47059	0.087369
34	120	2	308.3	167.1	141.2	229	73	1.9	21.56164	0.089708
27	120	3	213.4	116.9	96.5	248	58	1.7	20.43103	0.081435
37	120	4	191.6	103.5	88.1	202	56	1.6	17.71429	0.08881
24	160	1	181.9	99.5	82.4	218	56	1.5	17.05357	0.086283
39	160	2	258.7	129.5	129.2	267	65	2.0	20.06154	0.09908
30	160	3	169.3	94.1	75.2	224	43	1.7	21.09302	0.082911
29	160	4	160.3	88.1	72.2	251	44	1.6	19.45455	0.084346

Glasshouse data

treatment	P /leaf	P mg/g stem	total P mg/g	Total P/ plant	leaf DW g	stem DW g	Total DW
1	2.800	0.367	3.167	10.243	1.374	1.860	3.234
1	1.671	0.440	2.111	7.669	1.489	2.143	3.632
1	2.442	0.224	2.666	16.963	2.375	3.988	6.363
1	1.233	0.367	1.600	5.009	1.264	1.867	3.131
1	1.691	1.149	2.840	7.685	1.090	1.616	2.706
1	1.691	0.227	1.917	7.790	1.772	2.291	4.063
1	1.765	0.327	2.093	2.574	0.682	0.548	1.230
1	2.014	0.630	2.644	7.201	1.516	1.207	2.723
2	1.745	0.408	2.153	30.750	4.546	9.738	14.284
2	1.503	1.172	2.675	36.131	4.456	9.050	13.506
2	2.711	0.882	3.593	61.661	5.829	11.332	17.161
2	1.534	0.771	2.305	8.867	1.509	2.338	3.847
2	2.623	1.191	3.815	42.082	3.786	7.245	11.031
2	3.203	0.448	3.651	71.326	7.319	12.219	19.538
2	1.613	0.669	2.282	30.275	6.425	6.840	13.265
2	3.722	0.291	4.013	59.306	4.810	9.967	14.777
3	2.019	0.963	2.982	17.607	2.314	3.591	5.905
3	1.972	0.472	2.444	7.602	1.380	1.730	3.110
3	3.691	0.311	4.003	16.759	2.080	2.107	4.187
3	3.862	0.417	4.279	26.879	2.329	3.952	6.281
3	4.285	0.959	5.244	35.337	2.468	4.271	6.739
3	1.661	0.833	2.494	7.423	1.474	1.502	2.976
3	3.355	0.885	4.240	15.236	1.462	2.131	3.593
3	1.409	2.132	3.541	17.213	1.905	2.956	4.861
4	1.969	0.856	2.825	14.035	2.048	2.920	4.968
4	0.980	0.828	1.808	30.376	5.603	11.196	16.799
4	4.316	1.020	5.336	38.458	2.844	4.363	7.207
4	5.296	0.637	5.933	85.552	6.052	8.367	14.419
4	1.902	0.384	2.287	40.769	7.575	10.255	17.830
4	1.511	0.609	2.120	26.559	4.876	7.652	12.528
4	4.823	1.678	6.501	68.756	4.292	6.284	10.576
4	4.270	0.945	5.215	58.512	4.202	7.018	11.220