COTTON BUNCHY TOP (CBT) CHARACTERISTICS AND MODES OF TRANSMISSION

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Introduction

Cotton Bunchy Top (CBT), a relatively new disease, was first observed by growers in Australian cotton fields in the 1998-99 cotton-growing season. The disease has since been reported across New South Wales and Queensland, from the Macquarie Valley in the south to the Emerald region in the north. CBT is suspected to be spread by the cotton aphid (*Aphis gossypii*, Glover).

Symptoms of CBT include reduced plant height, leaf surface area, petiole length and internode length. Pale, angular patterns on the leaf margins are often observed with the remainder of the leaf blade usually dark green in colour. These darker leaves have a leathery and sometimes glossy texture when compared to healthy control plants. Typically, the pale angular patches in field-grown cotton turn red as leaves age. Boll development is also affected, with bolls often less than half the size of healthy bolls.

There are many cotton diseases, which show similarities to CBT, for example, 'terminal stunt' which was first reported in America (Texas) during the 1960's (Sleeth *et al.* 1963). Terminal stunt results in mottled leaves and shortened internode length although differences exist; the discoloration (tan to dark brown streaks) of the xylem is the most consistent symptom of terminal stunt, which has not been detected in any CBT infected plants. Other diseases that have some similarities with CBT include cotton blue disease, found in Africa and South America, cotton anthocyanosis, found in Brazil, and cotton leaf roll, found in Thailand, all of which are transmitted by the cotton aphid (Cauquil and Follin, 1983; Brown, 1992). However, no suite of symptoms, exactly matches those of CBT. In most cases a virus is suspected as the causal agent of these diseases, though not proven. The causal agent of CBT is unknown.

The first formal studies of the disease were initiated in the 1999-2000 cotton-growing season where physiological and morphological responses of cotton to CBT were recorded in the field. Following these observations, transmission studies were carried out to investigate if CBT was transmissible. This paper reports the first field studies of CBT, and modes of transmission found thus far. Further studies are currently underway and it is

hoped that in the future a better understanding of modes of transmission, epidemiology and the causal agent will be useful for improved management of CBT in the field.

Materials and Methods

Measurement of disease symptoms and plant performance

In order to establish the symptoms of CBT and effects on cotton growth, twenty plants (10 pairs of CBT affected plants and healthy plants) were selected from commercial cotton fields at each of three sites in NSW, "Hazeldene" in the Macquarie Valley, "Pindara" in the Namoi Valley and "Whynot" in the McIntyre Valley. Plant characteristics compared between CBT infected and healthy plants included the following: photosynthetic rate (at "Pindara" and "Hazeldene" only), plant height, mainstem leaf area, specific leaf weight and total number and dry weight of roots, stems, squares, flowers, bolls and leaves.

Transmission Experiments

(a) By Grafting

To test if the casual agent of CBT was graft transmissible, replicated grafting experiments were conducted at two sites, CSIRO Plant Industry, Adelaide, and CSIRO Plant Industry, Narrabri. In these studies transmission would be indicated by the development of CBT symptoms in healthy plants that had received a graft from a CBT affected plant, i.e. the disease agent crossed the graft.

Healthy cotton plants were grown in pots in glasshouses in Adelaide and Narrabri. Plants were divided into 2 treatments: (i) plants grafted with CBT affected terminals and (ii) plants grafted with healthy terminals. A 'wedge' graft technique was used. There were 40 CBT graft plants and 10 control plants at Narrabri and 5 of each at Adelaide. The regrowth beneath the graft was monitored over an 8 to 9 week period for CBT symptom development. Petiole and internode length, leaf surface area and branch height were also recorded on the regrowth.

In addition, tagged leaves on both infected and healthy cotton plants were monitored over a period of approximately 6 weeks to evaluate whether CBT symptoms remained constant or progressed in severity.

(b) By Aphids

Outbreaks of CBT have been strongly associated with the presence of high cotton aphid densities; with the worst instances found where there were 'hotspots' of aphids early in the cotton season. A glasshouse trial was conducted to test if the cotton aphid is a vector of CBT. Sixty healthy cotton plants were grown in individual pots. These were divided into 3 treatments (20 plants / treatment): treatment 1 plants inoculated with 100 aphids that had fed on CBT infected plants for at least 4 weeks (+aphids, +CBT), treatment 2 plants

inoculated with 100 aphids that had fed on healthy cotton plants (+aphids, -CBT), treatment 3 control plants with no aphids (-aphids, -CBT). After 10 days of feeding, aphid numbers were controlled using Confidor® (Imidacloprid) at 2 mL L^{-1}. Observations were made every two days for all 3 treatments for 5 weeks.

Six plants from each treatment were selected for comparison of petiole length, mainstem internode length, leaf chlorophyll content, leaf area and specific leaf weight in the regrowth beneath the graft.

(c) By Seed

Glasshouse experiments and a larger scale field trial were carried out to test if CBT could be transmitted via the seed of affected cotton plants. A total of 166 CBT seeds were harvested from the bolls of infected plants and 36 seed from healthy cotton. These were germinated in pots in the glasshouse and monitored regularly for the appearance of CBT symptoms over a period of 16 weeks.

A larger scale field trial was also set up at ACRI, Narrabri NSW, which consisted of three varieties, Delta pearl, Nucotn 37 and Siokra V16 that were known to be susceptible to CBT from other studies at ACRI. The treatments were (i) 1800 seeds harvested from plants affected with CBT and (ii) 1800 seeds harvested from healthy cotton plants. The seeds were sown into plots arranged in a randomised block design with four replicates. The trial was monitored closely for insects, especially sucking pests and controlled appropriately to decrease the chance of the CBT being accidentally transmitted into the experimental area. The plants were monitored weekly through the growing season for any symptoms of CBT.

Results and Discussion

Cotton Bunchy Top symptoms and plant performance

One of the most definitive characteristics of CBT is the development of the pale, angular mosaic pattern on the leaf margins, initially, and then the spread over the leaf (Figure 1). The pattern is visible on both the upper and lower leaf surfaces.

As the disease progresses within the plant, reduced leaf area, petiole length and internode length are also very prominent symptoms of the disease. Figure 2 shows an example of leaf area reduction as a result of CBT recorded in the field and in aphid and graft transmission experiments. The leaves also become thicker, as indicated by the increase in specific leaf weight and may have a leathery feel or glossy appearance. The leaves usually become darker in appearance, in areas unaffected by the leaf mottle, as a result of increased chlorophyll content (Figure 3). Boll size was also greatly reduced.

CBT infection can result in reduced photosynthetic rate as shown in Figure 4 from the data taken at Pindara. This reduction could have occurred as a result of small reductions in chlorophyll content in the areas of leaf mottle as the photosynthetic capacity of leaves is closely related to leaf chlorophyll content (Evans, 1989). Photosynthetic reactions may have also been reduced by some sort of interference by the causal agent within photosynthetically active leaf cells. Total plant photosynthesis may have been reduced due to reduced photosynthetically active leaf area, which in turn may have led to the reductions in boll, stem and root dry weight and hence yield.

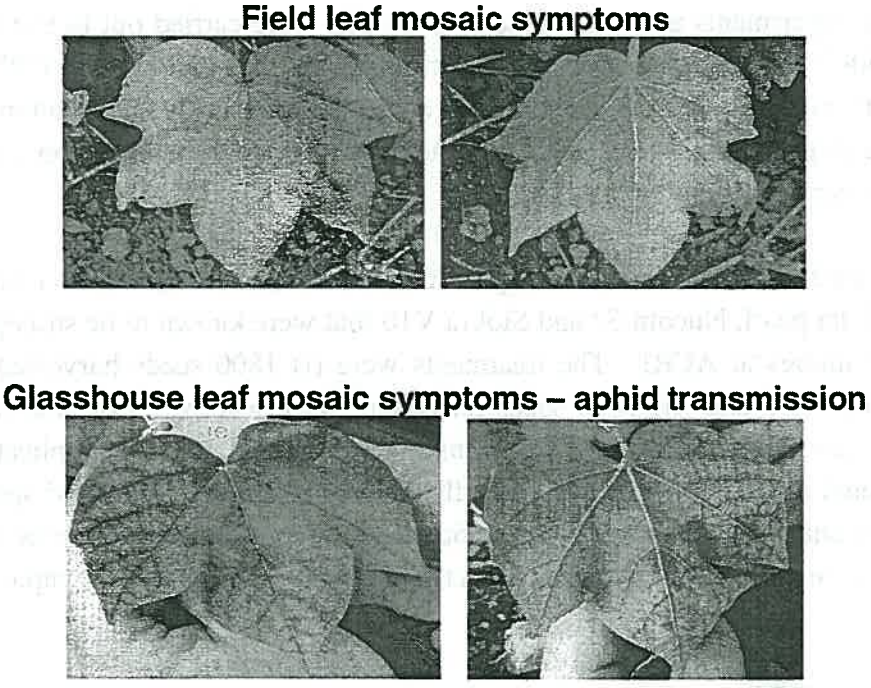

Figure 1. Symptoms of CBT: mosaic leaf pattern on upper and lower leaf surfaces in the field and leaf mosaic pattern in the glasshouse plants inoculated with aphids from CBT affected plants.

Furthermore, CBT symptoms appeared to progress in severity with age, as evaluated in the glasshouse plants inoculated via grafting. This was particularly the case with leaf development.

Some nutrient deficiency symptoms are similar to CBT, such as magnesium deficiency, which causes foliar reddening in the field with the main veins remaining green (Hodges, 1992). In the early phase of magnesium deficiency, the leaves have yellow patches, with the veins remaining green (Rochester, 2001), which can look similar to the CBT mosaic pattern. Inter-veinal chlorosis was noticeable in some of the glasshouse plants inoculated with CBT via grafting and aphid transmission (Figure 1). However, all the plants were regularly fertilised with magnesium sulfate and no magnesium deficiency symptoms were

evident in the control plants. It is possible that although magnesium was readily available, the CBT disease may inhibit its uptake by the plant.

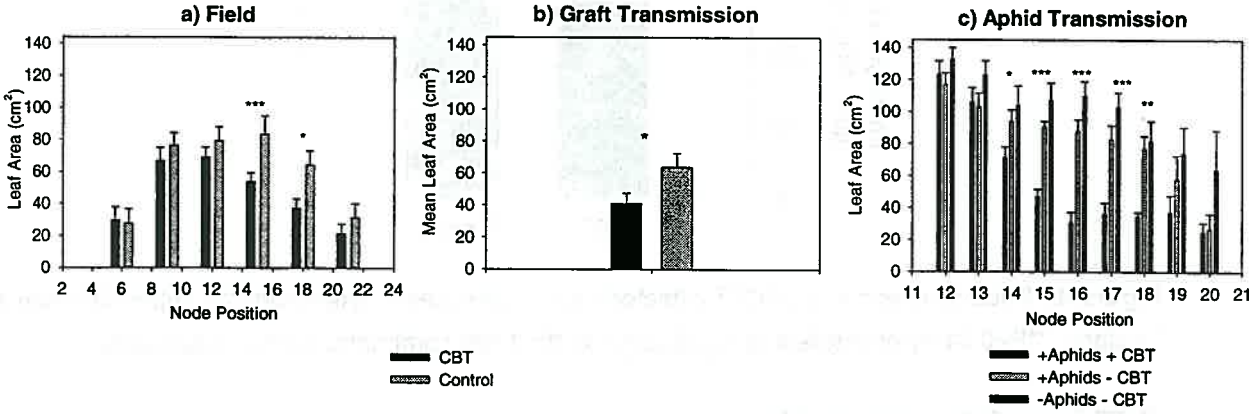

Figure 2. Leaf area measurements of CBT affected and healthy plants taken from a) natural field infection, b) graft transmission plants and c) aphid transmission plants. *P<0.05, **P<0.01 ***P<0.001 – symbols refer to significance of the t test comparing the two treatments in the field and ANOVA in the graft and aphid transmission experiments.

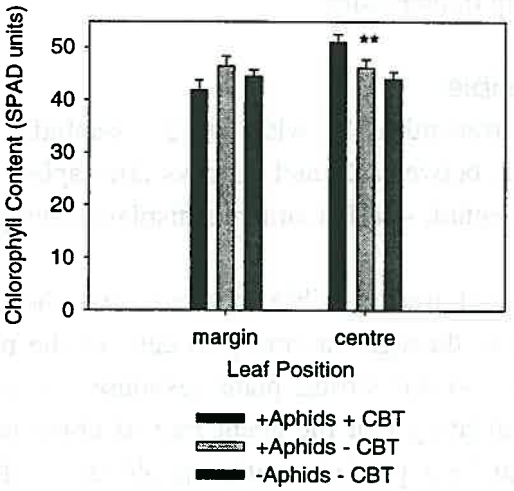

Figure 3. Chlorophyll content of CBT affected plants and non-CBT affected plants showing a significant increase in central +CBT leaf chlorophyll content and a slight decrease in +CBT leaf marginal chlorophyll content where the leaf mottle is predominant. **P<0.01 refers to significance of the ANOVA comparing the 3 treatments in the aphid transmission experiments.

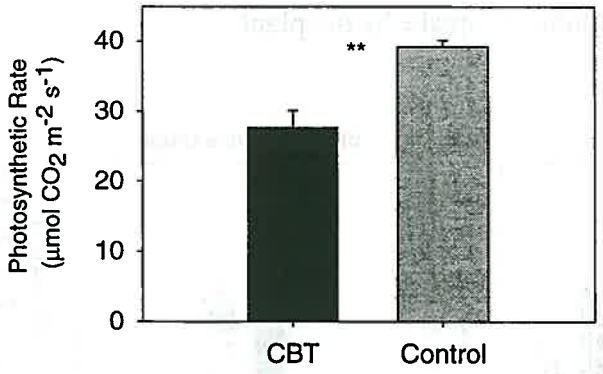

Figure 4. Photosynthetic rate of CBT affected and healthy plants taken from the cotton field site at Pindara. **P<0.01 symbol refers to significance of the t test comparing the two treatments.

CBT is graft transmissible

At Adelaide all five plants that were grafted with infected source material, developed CBT symptoms in the regrowth beneath the graft while the 5 control plants developed normal, healthy regrowth beneath the graft. At Narrabri, 38 of the 40 plants grafted with CBT affected terminals, developed CBT symptoms in the regrowth beneath the graft between 35 and 55 days post grafting, the control plants showed no symptoms. In the 2 plants that did not develop symptoms in the regrowth the grafted tissue died almost immediately after grafting probably preventing transmission.

CBT is aphid transmissible

CBT was clearly aphid transmissible with all 20 +aphid, +CBT treatment plants developing CBT symptoms, between 25 and 40 days after aphid transmission. No plants from the +aphid, -CBT or –aphid, -CBT treatments displayed symptoms.

That CBT is graft and aphid transmissible indicates that the causal agent of CBT is systemic, being able to move through the transport cells of the plant. The plants grafted with CBT affected scions showed strong plant responses in the branches that regrew beneath the graft point, indicating that the agent moved down into the rootstock via the phloem cells. Aphids also feed predominantly on phloem cells (Miles, 1987), further indicating that the causal agent resides in the phloem cells. Whether it occurs in other cell types is unknown.

A. gossypii is known to transmit over 50 plant viruses and is very common on cotton (Blackman and Eastop, 2000), so it was a likely vector candidate for CBT. However a number of other aphids species also feed or test feed on cotton including *Myzus persicae* Sulzer, *A. craccivora* Koch, *A. fabae* Scopoli and *Macrosiphum euphorbiae* Thomas (Blackman and Eastop, 2000) and need to be tested for CBT transmission. Furthermore, little is known about the characteristics of aphid transmission of CBT such as acquisition period, persistence or non-persistence of the disease and whether particular aphid instars

are required. Thus, this research opens the doors for much more follow up research into aphid transmission of CBT.

CBT is unlikely to be a seed transmitted disease

It is unlikely that CBT is seed transmissible as the glasshouse plants grown from CBT seed were observed for eight months, in which time no symptoms developed relative to the controls. The field trial was sown during the 2001/2002 cotton season and no symptoms developed over the season. Therefore, if CBT were seed transmissible, it would occur in a maximum of 1 in > 1966 seeds (the total number of plants observed that were grown from CBT seeds).

In many cases the rate of seed transmission of plant diseases is very low (less than 1%) (Pathipanawat *et al.* 1997 and Njeru *et al.* 1997). Thus continued testing would be required before ruling out the possibility of seed transmission of CBT, although, as there was no symptom development in 1966 plants tested, seed transmission is unlikely to result in serious CBT epidemics. Further testing of seed transmission may also be required once a molecular probe has been developed to test for CBT within the host plant. Although, no symptoms developed in the plants observed, it is unknown whether the disease may still have been present in the plant, perhaps at a very low concentration.

Conclusions

These initial studies answer basic questions, which will assist with CBT management. We envisage that management strategies will include management of alternative hosts of the vector/s and the use of resistant varieties.

Research is currently focussing on the determination of the casual agent and the development of a molecular probe to test for CBT. Isolating the pathogen of CBT has proved illusive thus far with no recognisable abnormalities found in CBT affected plant cells. However, differences in CBT DNA have been detected and a molecular probe is being developed from this to test plants or aphids for the presence of CBT, even though the identity of the pathogen or agent is unknown. In many disease cases, identification of the causal agent has proven difficult with no success after many years, particularly if the agent occurs only in small concentrations within the plant.

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