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Novel cotton germplasm with host plant resistance to twospotted spider mite

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ABSTRACT

Host plant resistance to twospotted spider mite (*Tetranychus urticae* Koch) in cotton is desirable to reduce crop damage, as well as to reduce or eliminate pesticide use targeted at this pest. A range of *Gossypium* genotypes were evaluated over two crop seasons and their resistance identified in terms of spider mite population development, leaf damage and effect on cotton yield and fibre quality. There were low to moderate numbers of spider mites on *Gossypium hirsutum* L. race lines and a *Gossypium barbadense* L. cultivar and extremely low numbers in accessions of *Gossypium arboreum* L., *Gossypium thurberi* Tod. and *Gossypium trilobum* (DC.) Skovst. compared with commercial cultivars. Mites reduced lint yield of the three *G. hirsutum* genotypes (Sicot 71, Siokra 24 and 81024-15) but had no significant effect on yield of the two *G. arboreum* genotypes, BM13H and Roseum $A_2$56 in either season. Yield loss was related to leaf area damaged by mites. BM13H, Roseum $A_2$56 and Sipima 280 were classified as mite-resistant, in terms of low mite numbers, low amount of leaf damage per mite and low yield loss per mite. In addition, the genotypes of *G. thurberi* (GOS5310), *G. trilobum* (GOS5332) and *G. hirsutum* race line (TX111) were also defined as mite-resistant, in terms of low mite numbers, low amount of leaf damage per mite, although there are no yield data because these genotypes are photoperiod sensitive and did not flower during the experiments. These mite-resistant genotypes can be candidates for the development of mite-resistant upland cotton cultivars.

1. Introduction

Twospotted spider mite (*Tetranychus urticae* Koch) is an important secondary pest of cotton (Leigh, 1963). Spider mites pierce the underside of leaves using their mouthparts and suck out the contents of mesophyll cells (Warabieda et al., 1997), which reduces the photosynthetic capacity of damaged leaves (Reddall et al., 2007) and can result in reduced yield and fibre quality (Wilson, 1993). Historically in many non-transgenic cotton systems, mites have been recurrent secondary pests, with outbreaks largely due to reduced mortality when natural enemy populations are reduced by pesticides applied against other pests. In Australia this was primarily due to the application of pyrethroid, organophosphate and carbamate pesticides targeted at the two lepidopteran pest species *Helicoverpa armigera* (Hübner) and *Helicoverpa punctigera* (Wallengren) (Wilson et al., 1998).

More recently transgenic Bt-cottons have been commercialised. The most recent of these, Bollgard II® (Monsanto Company, St. Louis, USA), which produces the Cry1Ac and Cry2Ab proteins toxic to *H. armigera* and *H. punctigera* has reduced insecticide use against these pests by up to 80% across the entire Australian industry (Constable et al., 2011). However, reduced spraying against *Helicoverpa* spp., has allowed populations of other pests, such as the green mirid (*Creontiades dilutus* Stal.), which were previously coincidentally controlled by *Helicoverpa* sprays, to increase. Green mirids now often require control, with up to three pesticide applications per season. The insecticides used against this pest are also generally disruptive of beneficial populations such as mite-predators and hence spider mites are still an important pest.

Hence, in both conventional and Bollgard II® cotton, chemical control of key pests, such as *Helicoverpa* spp. or green mirids, often reduces beneficial insect populations allowing spider mite populations to increase rapidly (Wilson et al., 1998), thus application of pesticides to control spider mites is often required. However these pesticides are often expensive and their use also selects for resistance in the mite populations. In Australian cotton systems, resistance in spider mite populations to bifenthrin (Herron et al., 2001), chlorfenapyr (Herron et al., 2004) and some organophosphates (Herron et al., 1998) has been reported.

Host plant resistance to spider mite in cotton is desirable, with the aim of reducing or eliminating pesticide use targeted against these pests. There is evidence for valuable host plant resistance to spider mites in the genus *Gossypium* due to a range of morphological (leaf shape, leaf hairiness) and biochemical characteristics (Wilson and Sadras, 1998). For instance, Wilson (1994b) showed that mite populations developed more slowly on okra leaf shaped upland cotton (*Gossypium hirsutum* L.) cultivars and this resulted in significantly reduced yield loss. High resistance to spider mites,

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Table 1
Details of genotypes used in the experiments and their descriptions.

Genotype	Species	Leaf shape	Description	References
Sicot 71	*G. hirsutum*	Normal	High yielding commercial cultivar	Reid (2003)
Siokra 24	*G. hirsutum*	Okra	High yielding commercial cultivar	Stiller and Reid (2005)
81024-15	*G. hirsutum*	Normal	Previously observed mite-resistance	Personal communication, Wilson and Reid
TX111	*G. hirsutum*	Normal	Previously observed mite-resistance	Personal communication, Stiller
Sipima 280	*G. barbadense*	Normal	Previously observed mite-resistance	Personal communication, Stiller
BM13H	*G. arboreum*	Normal	Previously observed mite-resistance	Personal communication, Stiller
Roseum $A_2$56	*G. arboreum*	Okra	Previously observed mite-resistance	Personal communication, Stiller
GOS5310	*G. thurberi*	Okra	Previously observed mite-resistance	Personal communication, Stiller
GOS5332	*G. trilobum*	Normal	Previously observed mite-resistance	Personal communication, Stiller

indicated by reduced population development and damage, has also been reported in *Gossypium barbadense* L. cultivars and moderate resistance in *Gossypium arboreum* L. genotypes (Schuster et al., 1972a,b,c; Trichilo and Leigh, 1985). Assessing host plant resistance must consider mite population development, plant damage and yield. For instance, Reddall et al. (2011) showed that smooth leaf genotypes had lower mite population development than near isogenic hairy leafed accessions, yet this did not prevent yield loss because the reduction in photosynthetic rate per mite was higher on the smooth leafed genotypes.

The objective of this study was to evaluate a range of genotypes from both tetraploid (*G. hirsutum*, *G. barbadense*) and diploid (*G. arboreum* L., *Gossypium thurberi* Tod. and *Gossypium trilobum* (DC.) Skovst.) species for their resistance to *T. urticae* by comparing mite population dynamics, leaf damage and effects of mites on yield and fibre quality.

2. Materials and methods

2.1. Design of field screening experiments

Two field screening experiments were conducted, one in the 2009–2010 season (Experiment 1) and the other in the 2010–2011 season (Experiment 2), at the Australian Cotton Research Institute, Narrabri, New South Wales, Australia. The genotypes of *G. hirsutum*, *G. barbadense*, *G. arboreum*, *G. thurberi* and *G. trilobum* used are detailed in Table 1.

Unless otherwise stated, the word 'mite' is used for the twospotted spider mite (*T. urticae*). The experimental design was a split-plot incorporating both mite-free and mite-infested plots with mite treatment as main plots and genotypes as sub-plots. The mite-infested plots were artificially infested with mites. Predators of mites were suppressed by regular application of non-miticidal broad spectrum insecticides to encourage mite population development (Leven et al., 2011). The mite-free plots were not infested and kept mite free through regular application of miticides. Each treatment was replicated four times. Seeds were direct sown with a cone seeder on 1-m row spacing on 21st October 2009 (Experiment 1) and 4th November 2010 (Experiment 2). Each plot consisted of three rows each 10 m long. Cotton was irrigated according to current practice (at a deficit of about 60 mm). Weeds were controlled by cultivation, hand hoeing and spot application of glyphosate as required.

2.2. Artificial infestation and pesticide treatments

Artificial infestation occurred during mid to late December using glasshouse-grown seedlings of the cultivar Sicot 71 which had been previously infested with mites (approx. 100 adult female mites per seedling). The infested seedlings were manually distributed across each plot at approximately five seedlings per metre. Artificial infestation was repeated three (Experiment 2) or four times (Experiment 1) to increase the efficacy of infestation.

After infestation, pesticides were applied weekly using a rotation of insecticides to suppress mites in mite-free plots, predators on mite-infested plots and control other pests such as *Helicoverpa* spp., green mirids or cotton aphids (*Aphis gossypii* Glover) in all plots (Table 2). For example, one application pattern (Rotation 1) was applied to plots the first week and then the next pattern (Rotation 2) was applied the second week. After the application of Rotation 4, Rotation 1 was applied the following week. This rotation of application was continued until cotton was mature as indicated by the presence of >60% of bolls open.

2.3. Population assessment of spider mites and analysis of leaf damage

Ten leaves from the third node below the terminal were harvested from the middle row of each plot on every sampling date. Wilson and Morton (1993) showed leaves in this position were the most likely to be infested with spider mites. Harvested leaf samples were collected in plastic bags and 2 L of washing solution (5% sodium hypochlorite and one drop of Tween®20 (ISI Americas Inc., Wilmington, USA)) was added to the bags and the mites washed off by gently shaking the bag for 30 s. An 80 μm microfilter sieve was placed on an empty jug and the sample was poured through the sieve to collect mites. This process was repeated three times. All the mites collected on the sieve were transferred onto blue-coloured filter paper (9.0 cm in diameter) using a Buchner funnel and the filter paper with mites placed in a petri-dish, which was sealed with tape so mites could be stored in a freezer for later counting. The number of eggs, nymphs, adult males and females were counted using a binocular microscope (Leica Wild M3C, Leica Microsystems GmbH, Wetzlar, Germany).

An additional three leaves from the third node below the terminal were randomly taken from the middle row of each plot to measure the leaf area damaged by mites. These leaves were scanned (HP Laser Jet M3027) in TIFF 300dpi colour mode and obtained images were analysed by the analySIS® LS Research Five 5.0 Professional Life Science Imaging System (Olympus Soft Imaging Solutions GmbH, Münster Germany). Areas of leaves damaged

Table 2
Rotations of pesticide applications used in the experiments.

Application pattern	Chemical ingredients
Rotation 1	
Mite-infested plots	150 g ha^{-1} chlorantraniliprole + 25 g ha^{-1} fipronil
Non-infested plots	150 g ha^{-1} chlorantraniliprole + 5.4 g ha^{-1} abamectin
Rotation 2	
Mite-infested plots	170 g ha^{-1} indoxacarb + 750 g ha^{-1} thiodicarb
Non-infested plots	170 g ha^{-1} indoxacarb + 5.4 g ha^{-1} abamectin
Rotation 3	
Mite-infested plots	13.75 g ha^{-1} deltamethrin
Non-infested plots	13.75 g ha^{-1} deltamethrin + 600 g ha^{-1} propargite
Rotation 4	
Mite-infested plots	95 g ha^{-1} spinosad + 25 g ha^{-1} fipronil
Non-infested plots	95 g ha^{-1} spinosad + 5.4 g ha^{-1} abamectin