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Evaluation of relative damage caused by two-spotted spider mite, bean spider mite and strawberry spider mite in cotton

FINAL REPORT: DAN1808

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Part 1 - Summary Details

CRDC Project Number: DAN1808

Project Title: Evaluation of the relative damage caused by two-spotted spider mite, bean spider mite and strawberry spider mite in cotton

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Part 3 - Final Report

Background

Three *Tetranychus* spp. have been identified in Australian cotton since at least the 1970's (Herron et al. 1998). They are *Tetranychus urticae* Koch (two-spotted spider mite-TSM), *Tetranychus ludeni* Zacher (bean spider mite-BSM) and *Tetranychus lambi* Pritchard and Baker (strawberry spider mite-SSM). Of the three species, TSM has been the most extensively studied in Australian cotton. TSM feeding damage is capable of reducing photosynthetic capacity and stomatal conductance which leads to decreased yield and fibre quality of cotton (Wilson 1993, Reddall et al. 2007). Management strategies and sampling protocols are based on TSM dispersal and colonisation behaviour in cotton (Wilson and Morton 1993, Wilson 1995).

To date, there has been limited research in Australia on BSM and SSM. Consequentially, the damage caused by both species has yet to be fully quantified and therefore its impact on Australian cotton has not been fully evaluated. The ecology of the spider mite complex in cotton landscapes has also not been extensively studied.

In recent years, the incidence of SSM in cotton has reportedly increased, while the incidence of TSM has declined (Herron and Marshall 2019). It was hypothesised that in the modern Australian cotton landscape of transgenic Bt cotton and reduced insecticide sprays, SSM was able to competitively displace TSM. This was primarily because the two species can be found together in the same fields. However, little is known about mite species composition and how this is influenced by ecological or environmental factors. Investigating mite species interactions may provide insight into the causes of the changing mite species complex in cotton.

The aims of this research are therefore to (1) evaluate the relative damage of each mite species on cotton and determine damage potential, (2) measure the development time and fecundity of each mite species to compare key life history traits, (3) investigate whether there are competitive behaviours between each mite species which may result in population suppression and/or displacement, (4) survey species composition of mite infested fields and evaluate whether the current mite sampling protocol is accurate for SSM infestation. The findings from this research will contribute to improved mite management in cotton by assisting cotton producers in making informed and suitable pest control decisions.

1. List of milestones and extent of achievement

Milestone 1. Review of literature (Appendix 1) - **Achieved**.

Milestone 2. Establishment and maintenance of mite colonies - **Achieved**.

Milestone 3. Establish methods to evaluate mite damage - **Achieved**.

Milestone 4. Design and conduct a glasshouse experiment to measure relative mite damage on cotton - **Achieved**.

Milestone 5. Design and conduct experiments to measure relative rates of population increase (R_o) measured for each species on excised leaves maintained under laboratory conditions - **Achieved in Part**. Partial completion of this Milestone was due to a delay in sourcing field populations of SSM and difficulty managing competing priorities in the final months of the project. Planned completion of this work in mid-2020 will facilitate publication of results.

Milestone 6. Design and conduct competition studies under two temperature regimes - **Achieved**.

Supplementary Milestone 7. Sample cotton fields in the Gwydir Valley NSW, to determine the relative abundance of the three mite species in crops during the 2018/19 season - **Achieved**.

2. Methods

2.1 Establishment of mite cultures

2.1.1 Source of mites

The TSM colony was established from a population that has been maintained on cotton in a glasshouse at the Australian Cotton Research Institute (ACRI) Narrabri NSW, since the 1990's. The BSM colony was sourced from a population maintained at the Elizabeth McArthur Institute (EMAI) Menangle NSW, and was reared on French beans (*Phaseolus vulgaris*). This culture has been maintained for approximately 40 years (Herron et al. 1998). Field populations of SSM proved difficult to find in 2018 most likely due to a combination of the drought, pesticide usage and a lack of taxonomic awareness among growers and agronomists. The SSM colony was eventually obtained in January 2019 by the author from commercial cotton fields near Croppa Creek in northern NSW (-29.103502, 150.324575). This necessitated further simultaneous species comparisons in subsequent experiments.

2.1.2 Mite rearing

The mite cultures were maintained on cotton plants and kept in a glasshouse at (27 ± 3.6°C) under ambient humidity and seasonal photoperiod. Cotton plants were raised in the glasshouse using the cotton cultivar 711 RRF with an imidacloprid seed treatment sown at a rate of approximately seven seeds per pot into a mixture of 70% potting mix, 30% perlite and 40g of slow release fertiliser (Osmocote®). Plants were watered as required and liquid fertilised (N-P-K: 12.0-1.4-7.0) once a week.

The glasshouse benches were lined with black construction plastic and binder clips were used to hold the plastic against the 5cm high lip around the bench. Four small pots were

inverted and a 45cm x 45cm steel tray placed on top of the pots. The pots of cotton seedlings were then placed inside the steel tray (Figure 1). Water was added to the bench and maintained up to the lip of the bench, creating a water barrier for reducing mite movement between trays. At certain times during experimentation an additional cage with the same dimensions as the tray and made of fine gauze fabric overlaying the fly wire was placed over the infested plants. This helped further reduce the transmission of mites by silk webbing and the spread of other pests such as whitefly and thrips.

The mite cultures described above were maintained at ACRI and provided mites used throughout this study. Specimens of male and female mites from each species culture were sent to Dr Owen Seeman at the Queensland Museum to confirm their taxonomic identification which was particularly important for SSM as it the most difficult species of the three to identify.

For experimental measurements, the adult female population was used as a reference point for how the mite population was performing as a whole (except for the Field Survey of SSM canopy distribution). Adult female mites can be distinguished by eye or with the aid of a 10x eye piece, whereas males are smaller and easily mistaken for juveniles without a stereomicroscope.

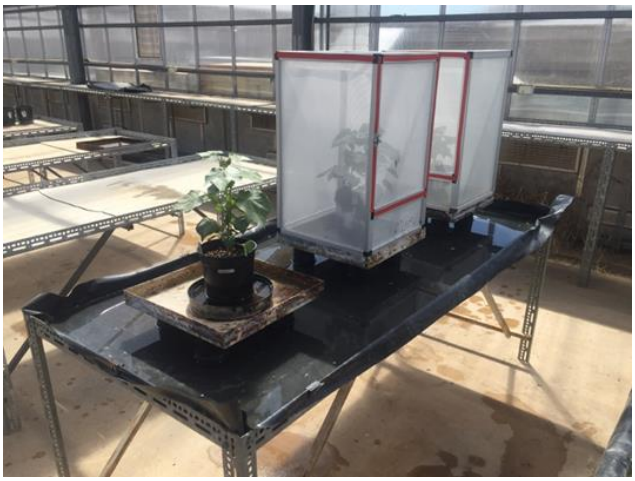

Figure 1. Spider mite cultures in the glasshouse.

2.2 Leaf damage experiments

Two separate experiments were conducted due to the delay in obtaining a colony of SSM until January 2019. Because they were based on the same experimental design, the following method applies to both experiments.

2.2.1 Measurement of leaf damage

Previous studies to determine mite feeding damage have utilised a wide range of tools from visual assessment to advanced equipment for measuring leaf gas-exchange (Škaloudová et al. 2006, Hussey and Parr 1963, Reddall et al. 2007). The most versatile and accessible option identified in the literature was a Leaf Damage Index (LDI). This is a comparative measure that allows fast assessment of leaf tissue (Hussey and Parr 1963) (Table 1). The LDI is based on a visual scoring system from zero (no damage) to five (high level of damage), with each integer representing 20% of leaf area damaged.

Table 1. Damage scoring system adapted from Hussey and Parr (1963).

Leaf Damage Index (LDI) score	% Leaf area damaged
1	1-20
2	21-40
3	41 – 60
4	61 – 80
5	81 – 100
6	Defoliated leaf

2.2.2 Experimental design of pot trials

Whole cotton plants were used to allow assessment of cumulative mite damage over multiple weeks, something not possible with excised leaves alone. Using whole plants also provided additional opportunities for measurements such as female population on the initially infested leaf. Cotton plants were raised in the glasshouse and arranged in a randomized block design. A fabric cage (45cm X 45cm X 75cm) was placed over each pot to reduce transmission of mites and other pests between plants (Figure 2). Two female mites were transferred to the 3rd or 4th fully expanded leaf from the terminal and the leaf tagged as the L1 leaf (Reddall et al. 2004). Control plants had no mites. In total, there were 30 replicates for TSM and BSM and 20 replicates for SSM. After one week, the LDI score and female population on the L1 leaf were recorded and measurements taken weekly in each of the following four weeks.

Figure 2. Damage experiments used fine mesh cages to contain mites and reduce incidence of other glasshouse pests on test plants.

2.2.3 Data analysis

The data from the two damage experiments were collated for the purposes of analysis. The key assumptions for the analysis were that each plant had either no mites (control) or mites of only one species. It also assumed a randomised block design, with repeated measures per replicate.

A mixed model approach was used to fit a model for the repeated measures. Alternative correlation structures were examined and a structure chosen for the model using the

Akaike information criterion (Akaike 1974). Random effects were fitted for experiments 1 and 2 and for plants within each species. The residuals were plotted and were satisfactory. The model

was fitted and had terms for week, species, the female population nested within species, and interactions. The full model was then simplified, if possible, by removing non-significant effects. Then the interaction between species and week was sliced to determine where each species was different across each week. Predicted means were compared using the Tukey method least square means.

To test whether week or number of females was more important, recursive partitioning was conducted using the C Tree library in R (R Core Team 2018). All analyses were done using SAS software Proc Mixed version 9.4 (SAS Institute, Cary NC) in R 1.1.463 (R Core Team 2018).The figures in the results represent predicted values from the model described above. The most appropriate model excluded week 1, as this was a baseline measurement before mites were transferred and plants within each mite treatment were not significantly different (BSM -SSM, $t_{(196.7)} = -0.19$, $p = 0.98$), (BSM – TSM, $t_{(197.2)} = 0.27$, $p = 0.96$), (TSM – SSM, $t_{(197.3)} = 0.42$, $p = 0.91$). Where possible, t-test statistics are providing between means. Otherwise, tables of multiple comparisons were provided.

2.3 Competition experiments

The competition experiments were designed to determine whether mite species experienced population suppression or displacement when in combination with another mite species on the same plant. Two glasshouse experiments were performed, each comprising six paired combinations (treatments). Treatments were replicated ten times in each of the two experiments.

2.3.1 Experimental design of pot trials

In the first experiment, plants were raised in the glasshouse at 30.1 ± 4.5°C, ambient humidity and seasonal photoperiod. Two seeds of 711 RRF (hereafter non-B3) were sown into small pots. Once each seedling had reached the 3rd or 4th true leaf stage, two adult female mites from each of two species cultures were transferred to the first fully expanded leaf in each paired combination (treatment) (Table 2).

Table 2. Competition Experiment 1 treatments at 30.1 ± 4.5°C.

Plant treatment	Adult female mites
TSM/BSM	2 TSM, 2 BSM
TSM/SSM	2 TSM, 2 SSM
BSM/SSM	2 BSM, 2 SSM
TSM alone	2 TSM
BSM alone	2 BSM
SSM alone	2 SSM

The second experiment was conducted to validate the results from the first experiment at a lower temperature (26 ± 1.1°C) and to investigate cultivar variability. The non-B3 cultivar used was 711 RRF and the B3 cultivar used was 714 B3F. Combinations of species (treatments) are shown in Table 3. After 14 days, the number of adult female mites of each species was counted using a stereomicroscope at 7.5x magnification. Using the cotyledons

as a starting point, each leaf was removed from the plant one node at a time and the number adult females on each leaf were recorded.

Table 3. Competition Experiment 2 treatments at 26 ± 1.1°C.

Plant treatment	Cultivar	Adult female mites
TSM/SSM	Non-B3	2 TSM, 2 SSM
TSM/SSM	B3	2 TSM, 2 SSM
TSM alone	Non-B3	2 TSM
SSM alone	Non-B3	2 SSM
TSM alone	B3	2 TSM
SSM alone	B3	2 SSM

2.3.2 Data analysis

The two competition experiments were analysed separately as they were performed at different temperatures. The analyses for both experiments were conducted in the same way except where stated. Due to the sparse data on nodes 7 and 8, these data were omitted from the analyses. The two cotyledons (c1 and c2) were combined, leaving just c1 as a total in the analysis. Random effects were fitted for the replicates and species was nested within each treatment. A square root transformation of the data was required to normalise the data.

After fitting the full model and testing for significant effects, it was then simplified, if possible, by removing non-significant effects. Significant interactions were then sliced to determine how they arose. Predicted means were compared using the Tukey method least square means with an alpha = 0.05. For experiment 2, the cultivars of non-B3 and B3 were analysed together and separately with the same method. All analyses was performed using SAS software Proc Mixed version 9.4 (SAS Institute, Cary NC) in R 1.1.463 (R Core Team 2018). The results are presented as the actual means rather than predicted values from the model, with significant results derived from the analysis.

2.4 Field survey

A field survey of SSM canopy distribution was conducted during February 2019. Field infestations of TSM and BSM were not evident over the duration of the project and therefore field survey data for these species is not available to report.

2.4.1 Field selection

Fields with mite infestations were sought for surveying mite distribution and species composition. A population of SSM was reported in cotton fields near Croppa Creek NSW (-29.103502, 150.324575) in the 2018/19 season. The cotton crop surveyed and sampled was chosen due to its potentially higher infestation and close proximity to remnant vegetation which was considered to be the source of SSM.

2.4.2 Survey method

The canopy survey was based on the current industry standard for mite sampling (Williams 2018). This involved walking along rows, taking main stem leaves from the 3rd, 4th or 5th node down from the plant terminal and recording presence or absence of mites from any life stage. The sample size required varies on the field size, but is usually three to four rows, collecting 50 leaves from 50 plants (Williams 2018). The modified method used was the following:

1. Randomly selected sampling rows.
2. Walked 10 metres in from the edge of the field.
3. Walked along the row and sampled main stem leaves from the 3rd- 5th node (top), then approximate the middle and bottom portions of the canopy.
4. Plants were sampled every few steps from either side of the row.
5. Leaves were placed in large paper bags marked with each section of the canopy.
6. After a total of 30 plants were sampled from a row the leaves were stored in an esky for transport to the laboratory where they were then stored in a cool room at 10°C.
7. Leaves were examined separately using a stereomicroscope to count all mites of an active life stage on either side of each leaf.

2.4.5 Data analysis

The total number of mites counted on each leaf, from the three canopy sections were compared using a one-way ANOVA in SPSS Statistics version 25 (SPSS, Inc., Armonk, NY).

2.5 Life history trait analysis

Previous studies of TSM and BSM life history varied markedly in methodology and experimental conditions (Carey and Bradley 1982, Silva 2002, Miyazaki et al. 2013). Consequently, it was difficult to compare these studies. Only one study was identified in the literature involving *T. lambi* (SSM) (Bonato and Gutierrez 1999). There is however considerable doubt over the taxonomic identification of SSM and whether or not multiple species were identified as SSM (Seeman and Beard 2011). Therefore, side-by-side comparisons are important for to quantifying life history traits of the three mite species found in Australian cotton.

3.5.1 Mite development

The life history traits of each species were recorded using a modified leaf disc technique (Wilson 1994). For the leaf discs, cotton leaves were removed from cotton grown in the glasshouse. In the laboratory, discs were cut from leaves using a sterilised 13mm cork borer and placed adaxial side down on floral foam cylinders saturated with sterilized water (Figure 3). This served to hydrate the leaf disc and minimise the chance of mites escaping.

Mites were transferred to the leaf discs the same day of preparation. A single adult female mite from general rearing colonies of TSM, BSM and SSM was transferred to each leaf and allowed to oviposit for 24 hours after which time the female mites were removed and the eggs individualized so that there was one egg per leaf. Each petri dish was arranged inside a plastic container with paper towel taped on as a lid to reduce drying out of leaf discs. The

container was maintained in a controlled temperature room at 25 ± 2°C. Mite survival and development was observed every day until adulthood.

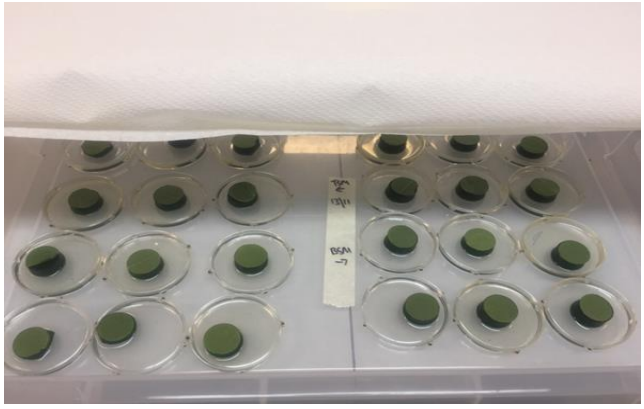

Figure 3. Experimental method for life history analysis.

2.5.2 Mite fecundity

Female fecundity was evaluated by pairing adult female mites with a male for 24 hours, after which the males were removed. The egg lay per day was recorded until the female mite died. This was completed for TSM and BSM but was not completed for SSM.

2.5.3 Data analysis

The time taken from egg to adult for each species was compared using a Kruskal-Wallis Test due to the low sample number of SSM. A significant difference (χ^2 (2, n = 142) = 29.64, $p < 0.001$) across the species was detected and subsequent Mann-Whitney U tests conducted to determine differences between each mite species pair. A Bonferroni adjustment was made to the alpha values due to multiple comparisons. A t-test was performed on the average egg lay per day and total egg lay for TSM and BSM. All analysis was conducted in SPSS Statistics version 25 (SPSS, Inc., Armonk, NY).

3. Results

3.1 Leaf damage experiments

Mean LDI scores are shown in Figure 4. The mite species, week and interaction between species and week, were all significant effects (week: $F_{(3, 133)} = 42.47$, $p < 0.0001$) (species: $F_{(3,134)} = 44.44$, $p < 0.0001$) (species x week: $F_{(9,155)} = 10.67$, $p < 0.0001$). From week two to week five, the TSM mean leaf damage was significantly higher than that of the other mite species. Furthermore, at the end of the experiment, 33% of leaves with TSM had defoliated. No defoliation was observed when BSM or SSM fed on the plants. Damage in the control (no mite) was due to the incursion of thrips (*Thrips tabaci* Lind) or environmental factors affecting the leaf quality. The level of damage also increased over time for the control as the leaves aged and the factors previously mentioned accumulated.

The difference in damage level between TSM and the other mite species also increased in each week, reaching a mean score of 3.9 ± 0.14 or approximately 60% of the leaf damaged at week five. The mean damage score for SSM was not significantly different from the no mite control from week two to week five, with a mean score of 1.3 ± 0.31 and 0.6 ± 0.28,

respectively, at week five. Only in week four is the BSM leaf damage significantly higher than SSM ($t_{(109.4)} = 2.74$, $p = 0.03$). Otherwise, at each week BSM scored significantly higher than the no mite control, resulting in a mean score of 2 ± 0.27 at week five.

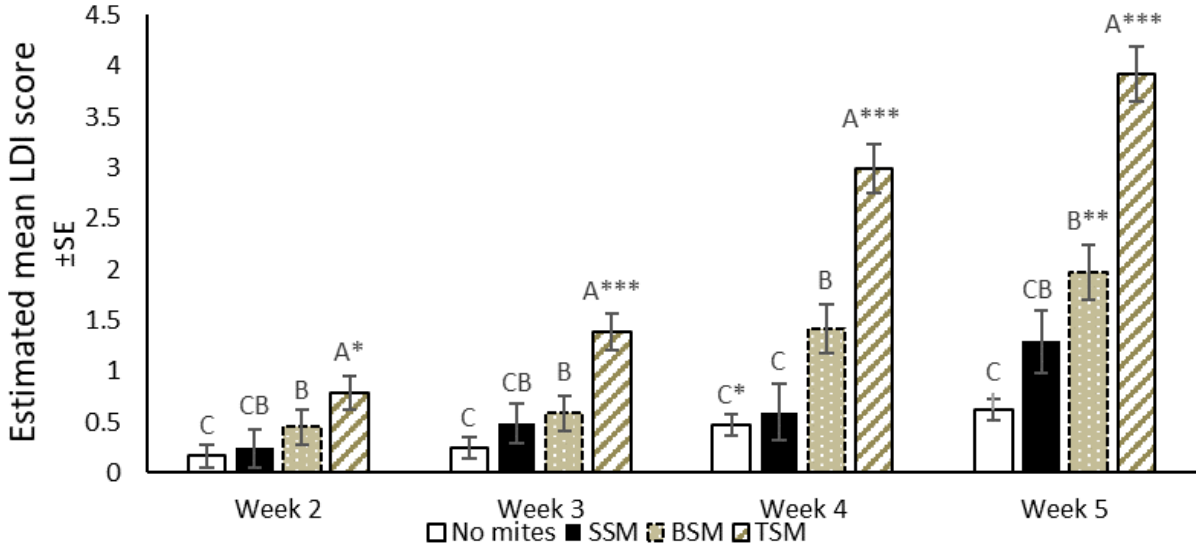

Figure 4. The estimated LDI score each week, excluding week 1. The same letter within each week are not significantly different at the $p < 0.05$ level. * = $p < 0.01$, ** = $p < 0.001$, *** = $p < 0.0001$.

The density of female mites in each species is shown in Figure 5. The mean number of adult female mites on the L1 leaf peaked at week three for TSM (19 ± 3) and BSM (36 ± 4). Whilst SSM also increased from week two to week three, the number of adult female SSM females peaked one week later at 27 ± 4. Although female mite numbers were highest in weeks three (TSM and BSM) and four (SSM), damage peaked in week five for all mite species. Despite TSM having the lowest number of female mites, plants with TSM sustained the highest levels of leaf damage. After the number of adult female mites peaked for each species, they all subsequently declined in the following weeks. BSM had the greatest decline with female number reduced to 4 ± 1 by week five. SSM had a more gradual decline, with numbers of females reduced by 50% to 13 ± 3.

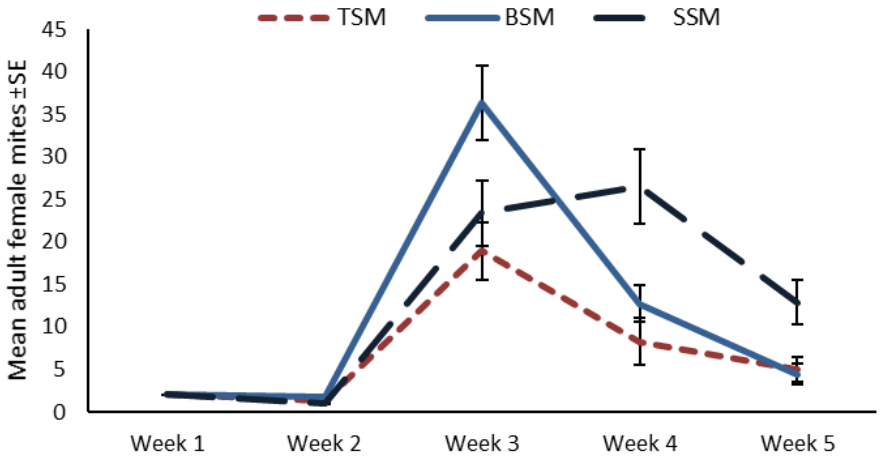

Figure 5. The mean number of adult female mites on the L1 leaf for each species.

3.2 Competition experiments

3.2.1 Experiment 1 – pair combinations among all three species on one cotton cultivar

Across all mite species and treatments it was found that treatment ((type of pairing); $F_{(5, 520)} = 4.74$, $p < 0.001$)), species ($F_{(3, 514)} = 2.64$, $p = 0.046$) and the node mites resided on ($F_{(5, 18)} = 4.82$, $p < 0.0001$) were all significant factors. The mean number of adult females per plant for each mite species and mite pairing indicates a trend toward lower numbers of both BSM and SSM when in combination with TSM. In contrast, TSM performed equally well alone or in any paired combination (Figure 6a-c).

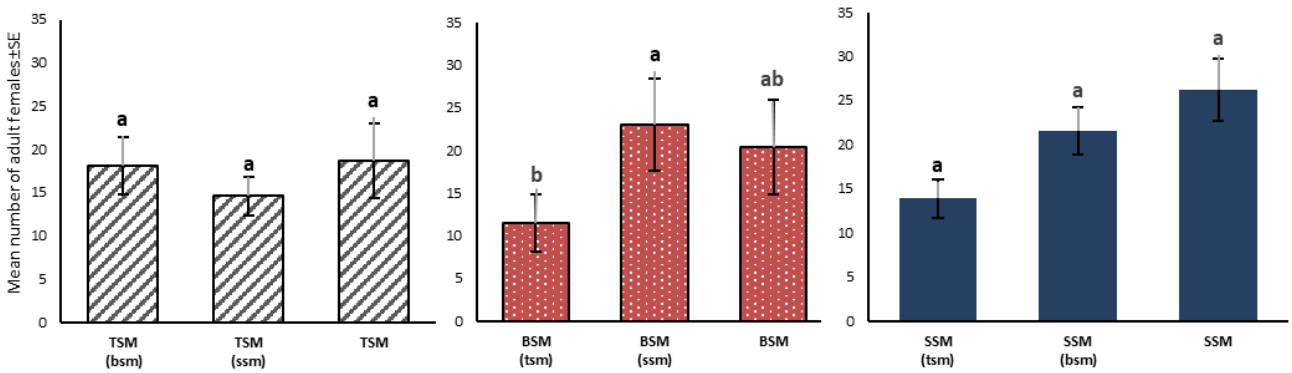

Figure 6a-c. Each mite species mean total count per treatment at 30.1 ± 4.5°C. a) Mean number of adult female mites per plant for TSM in each treatment remaining relatively unaffected by pairings with other mites. b) The mean number of adult female BSM significantly lower when paired with TSM. c) The mean number of adult female SSM varying across treatments but no significant difference. Columns with the same letter are not significantly different ($p < 0.05$).

There was no significant effect of species pairing on the number of adult female TSM ($F_{(2, 161)} = 0.06$, $p = 0.94$) (Figure 6a) or between the interaction of species pairing and the number of female mites on each node of the plant ($F_{(12, 149)} = 0.72$, $p = 0.73$). The species pairing for BSM was a significant effect ($F_{(2, 151)} = 3.73$, $p = 0.026$). BSM had significantly lower adult females when paired with TSM than when with SSM ($t_{(166.6)} = -2.70$, $p = 0.021$) (Figure 6b). The number of adult females on BSM alone to BSM paired with TSM was not found to be significantly different ($t_{(165.5)} = -0.99$, $p = 0.59$) (Figure 7a-b).

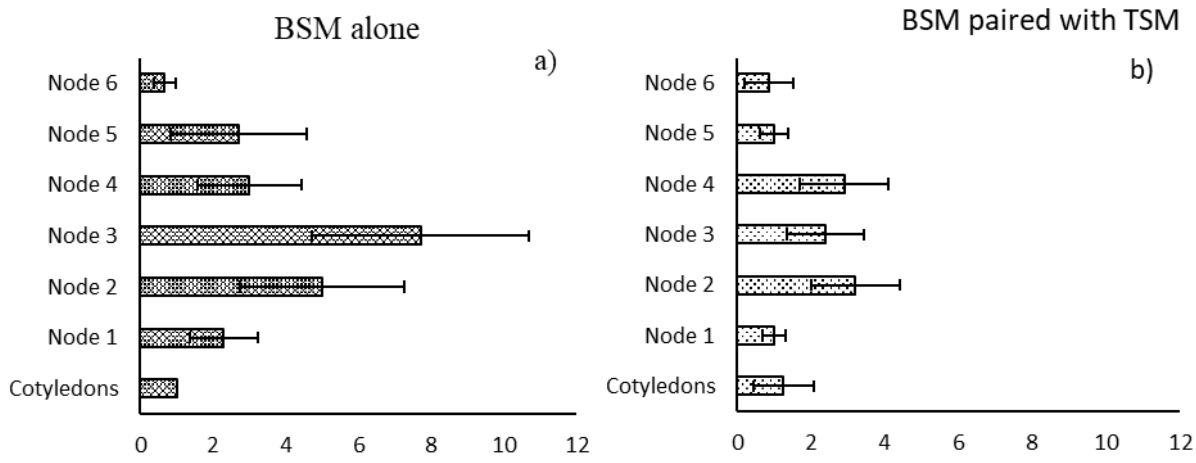

Figure 7a-b. Mean number of adult BSM females ± SE from each cotyledon and node of the plant.

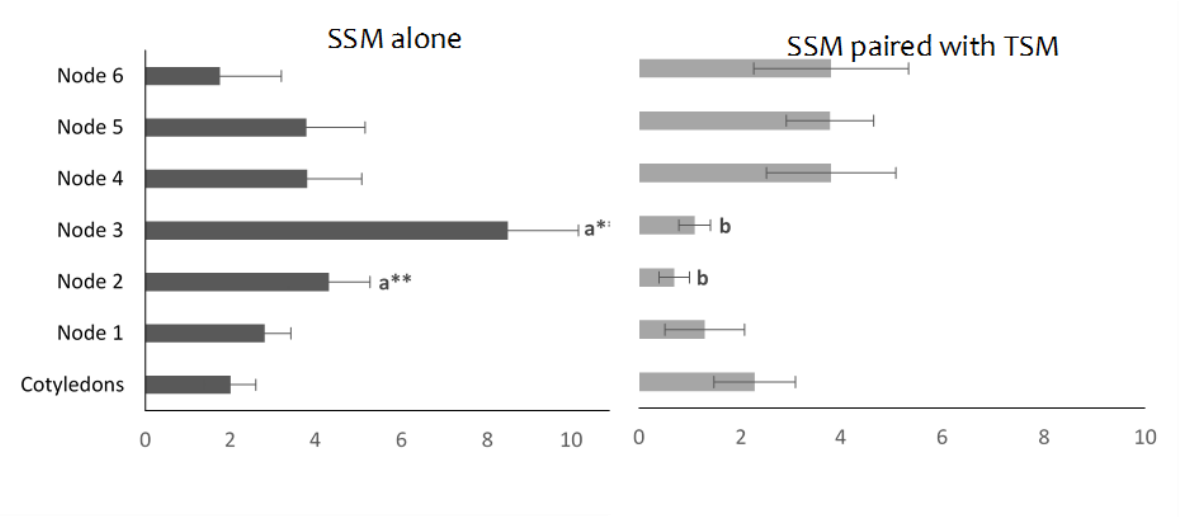

Figure 8a-b. Mean number of SSM adult females from each cotyledon and node of the plant. Nodes 2 and 3 had a significantly higher number of SSM females when alone than when paired with TSM (Node 2: $p < 0.001$, Node 3: ($p < 0.0001$). Columns with the same letter are not significantly different ($p > 0.05$).

There was also no significant difference between SSM when alone and in paired combinations ($F(2, 146) = 2.89$, $p = 0.059$) (Figure 6c). However, there was a significant interaction between female numbers on nodes and species pairing ($F(12, 142) = 3.45$, $p = 0.0002$) and SSM was the only species to show such an interaction (SSM ($F(12, 153) = 0.58$, $p = 0.84$, TSM ($F(12,149) = 0.72$, $p = 0.73$).

When the SSM data was separated by plant node, SSM paired with TSM had significantly lower numbers of adult females on node 2 ($t_{(141)} = -3.80$, $p = 0.0006$) and 3 ($t_{(141)} = -4.97$, $p < 0.0001$) than when SSM was alone (Figure 8a-b). When SSM was alone, node 3 (the node where the mites were placed initially) had the highest number of adult SSM females (8.5 ± 1.7). Both BSM ($F_{(6, 164)} = 3.07$, $p = 0.0071$) and TSM ($F_{(6,162)} = 2.48$, $p = 0.025$) were generally not distributed uniformly across nodes (Figure 7a-b). However, the distribution of BSM and TSM when paired with other species did not change significantly from when species were alone. In contrast, lower numbers of female SSM on nodes 2 and 3 when paired with TSM suggests that SSM may be at a competitive disadvantage in combination with another mite

species. Generally, higher numbers of mites of each species were around the nodes they were initially placed upon (Nodes 2-4) and, in the case of BSM, numbers were lower on cotyledons.

3.2.2 Experiment 2 - comparison of SSM & TSM alone and combination on two cotton cultivars

Comparisons between TSM and SSM (including both cultivars) in each paired comparison and when alone showed that the mean number of adult TSM females was significantly higher than SSM adult females ($F_{(1,474)} = 59.21$, $p < 0.0001$). The mean number of SSM adult females between treatments was not significantly different when either paired with TSM, alone or on either cultivar ($F_{(1, 474)} = 0.11$, $p = 0.74$) (Figure 9).

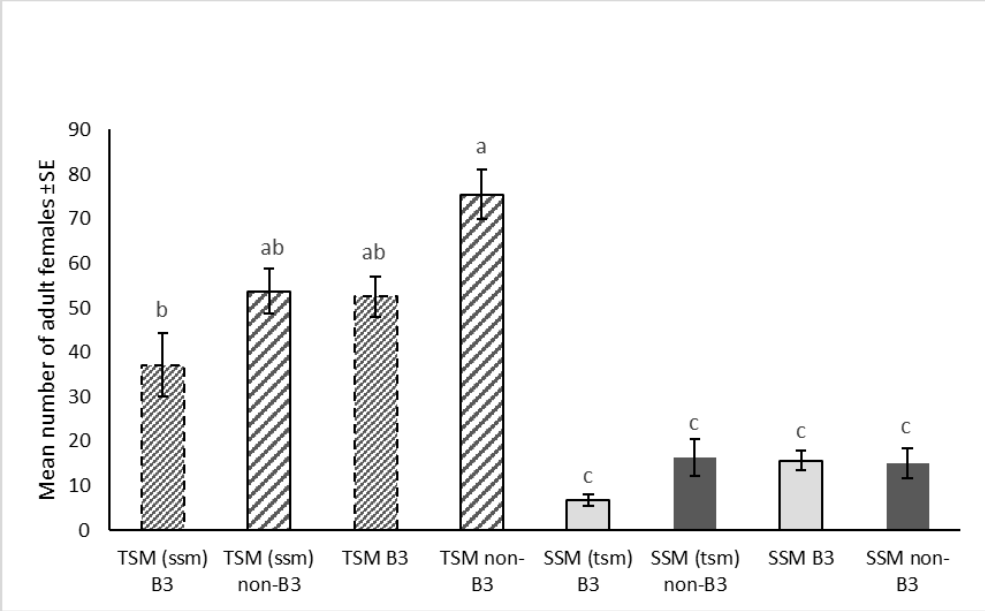

Figure 9. Competition between TSM and SSM on two cultivars at 26 ± 1.1°C. Columns with the same letter are not significantly different ($p > 0.05$).

There was a significant effect of cultivar on overall mite numbers ($F_{(1,475)} = 7.97$, $p = 0.005$) and significant two- and three-way interactions between cultivar and node, treatment and mite species. However, overall the non-B3 cultivar of cotton had more mites present than the B3 cultivar ($t_{(1,475)} = -42.57$, $p = 0.0104$). There was a significant difference between the mean number of TSM adult females alone on non-B3 and when paired with SSM on B3 ($t_{(475)} = 5.15$, $p < 0.0001$) (Figure 10).

There were no significant differences in the mean numbers of adult female mites of SSM between nodes when paired with TSM and when SSM was alone. Nevertheless, TSM had more adult female mites on every node in each treatment and significantly more on node 1 (B3) ($t_{(474.1)}$, $= -2.81$, $p = 0.02$), node 3 (non-B3) ($t_{(474.1)} = -3.47$, $p = 0.003$), node 4 (non-B3) ($t_{(474.1)} = -4.75$, $p < 0.0001$), node 5 (non-B3) ($t_{(474.1)} = -3.12$, $p = 0.01$, node 6 (non-B3) ($t_{(474.1)} = -2.74$, $p = 0.03$) when paired with SSM.

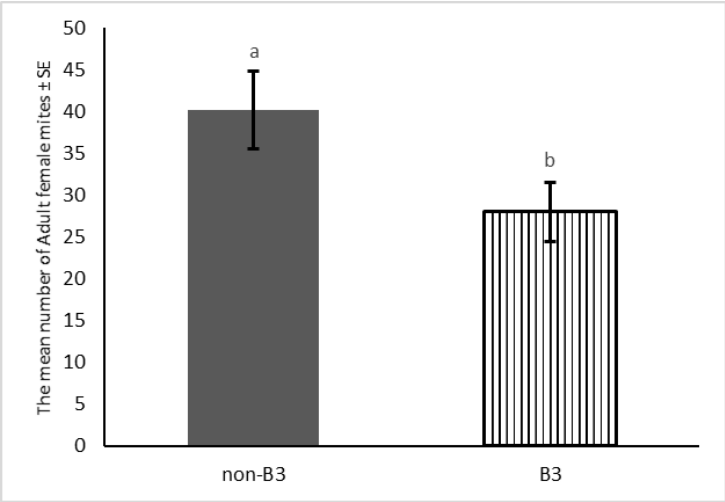

Figure 10. Mean total number of female mites (both TSM and SSM) on each cotton cultivar. Columns with the same letter are not significantly different ($p > 0.05$).

3.3 Field survey

The late season distribution of SSM (number of mites at any stage) within the cotton canopy at three levels was not significantly different. The mean number of mites on the top, middle and bottom main stem leaves was very similar at 52, 51, 51 mites/leaf respectively (Figure 11). The top level represents the position on plants where leaf sampling of main stem nodes is recommended.

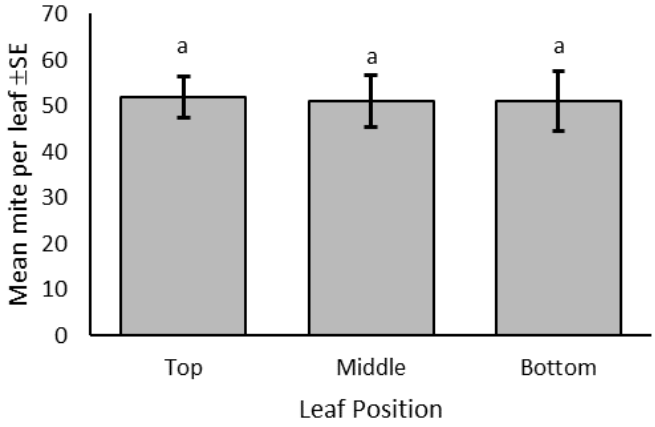

Figure 11. Late season canopy distribution of SSM at three levels within the canopy, n = 30 for each level. Columns with the same letter are not significantly different ($p > 0.05$)

Field sampled leaves appeared to have reddening on the veins which was not observed under glasshouse conditions. However, there was little to no visible damage on the adaxial surface of the leaf. Whilst conducting the collection of leaves and subsequent counting of mites in the laboratory, two adult female BSM were also detected in the same field as SSM.

The farm advisor stated that sampling conducted had estimated 8% of plants infested with one or two mites per leaf (R Holmes 2019, personal communication, 9th January 2019). On the 15th of January 2019, fipronil was applied for the control of mirids (*Creontiades dilutus*) and the author conducted the survey on the 26th of February 2019. The results from that

survey indicate 100% of plants infested and with a mean of more than 50 mites per leaf, with some leaves having in excess of 100 mites.

Since there was no significant difference between levels within the canopy, sampling from the top level of the canopy (3rd, 4th, 5th main stem nodes) where sampling is recommended, would not affect accurate sampling using the current sampling protocol. However, the results are only representative of a late season infestation of SSM and therefore early season surveillance would be recommended.

In the case reported herein, the crop only exhibited minor whitening along the veins on the underside of the leaves, as SSM was the dominant species present. The agronomist's connections with a research extension officer, also facilitated communication with the author and was able to support the conclusion that the species present was SSM and that the damage did not justify control. There are however reported cases where mite populations of unverified species are being controlled (Herron and Wilson 2016). The findings from the damage experiments also corroborate what was observed in the field. That is, SSM appears unable to cause visible damage to the upper leaf surface from feeding on the underside of the leaf, typically no necrotic spotting or evidence of cell death and no defoliation.

3.4 Life history trait analysis

3.4.1 Mite development

The development time in days from egg to adulthood was found to be significantly different between each species of mite (χ^2 (2, n = 142) = 29.64, $p < 0.001$) (Table 4). TSM development was fastest from egg to adulthood (mean = 11.39 days) and was significantly faster compared with to BSM (mean = 12.27) (U = 663, $p < 0.001$). BSM development time was not significantly different to SSM (mean = 13.67 days) after a Bonferroni adjustment (U = 30.5, p = 0.02). SSM had the slowest development to adulthood, significantly longer than TSM (U = 74.5, p = 0.001) (Table 4).

Table 4. Development time from egg to adult in days for each mite species. 25 ± 2°C.

Species	Mean development time (days)[1]
TSM (n = 110)	11.39 ± 0.08[a]
BSM (n = 26)	12.27 ± 0.2b[c]
SSM (n = 6)	13.67 ± 0.62[c]

[1]Means followed by the same letter are not significantly different at $p > 0.05$.

Table 5. Average number of eggs laid per day and total number of eggs laid for TSM and BSM at 25 ± 2°C.

Species	Mean egg lay/day ± SE[1]	Total eggs laid ± SE[1]
TSM (n = 25)	6.3 ± 0.3a	60.2 ± 4.5[a]
BSM (n = 13)	5.3 ± 0.4a	68.2 ± 9.8[a]

[1]Means and totals within columns followed by the same letter are not significantly different at $p > 0.05$.

3.4.2 Mite fecundity

The mean number of eggs laid per day per female by TSM and BSM was not significantly different, with average fecundity of 5-6 eggs per day ($t(36) = 2$, $p = .053$) (Table 5). Similarly, the total eggs laid for TSM and BSM were not significantly different ($t(17.05) = -0.75$, $p = 0.465$) with average lifetime fecundity ranging between 60 and 68 eggs. Fecundity of SSM was not recorded due to poor mite survival.

4. Discussion and conclusions

4.1 Comparative assessment of damage potential of three mite species in cotton

The first aim of the project was to quantify damage caused by each mite species on cotton to provide an evidence-based assessment of the relative damage potential of each mite species. A more targeted mite control strategy in cotton would be highly beneficial and result in reduced spray applications, slower resistance evolution and a lower cost of production.

Damage experiments demonstrated that despite TSM having the lowest peak density of female mites, plants with TSM sustained the highest levels of leaf damage, causing twice the damage of BSM and three times the damage of SSM. By week five, 33% of leaves on plants infested with TSM had defoliated. No defoliation was observed for either BSM or SSM. In addition, the damage caused by SSM was never significantly different from control plants where mites were excluded.

Key messages

- **These results provide an evidence-based foundation for claims about differential damage potential of BSM and SSM compared with TSM in cotton. Results demonstrate that SSM and BSM populations have the capacity to increase faster than TSM populations. Nevertheless, the comparative severity of damage for each species over time indicate SSM and BSM are unlikely to cause defoliation under ideal conditions. Similar dispersal behaviour was recorded for each spider mite which may indicate that movement within the cotton crop is similar.**
- **These results demonstrate that economic thresholds developed for TSM should not be applied to BSM and SSM infestations and highlight the importance of correct species identification of mites present within fields to enable appropriate management decisions. Assumptions of species composition and the practice of prophylactic spraying can unnecessarily increase resistance selection pressure and cost of production while reducing the population of beneficial insects.**

4.2 Species competition

The first competition experiment involving three mite species showed that the presence of TSM resulted in a reduction in the total number of female BSM when compared with BSM alone. In contrast, the presence of TSM did not result in a significant reduction in female SSM when compared with SSM alone. There was also an effect on mite distribution. The nodes with the most females of SSM alone were significantly reduced when paired with TSM. In contrast, TSM remained relatively unchanged regardless of position or mite pairing on the plant.

The difference across plant nodes for SSM paired with TSM was evidence of within-plant displacement by TSM. Mite adaptations to host plant defences can entail metabolic

resistance to volatile compounds and suppression of jasmonic and salicylic acid pathways linked to plant defence (Sarmento et al. 2011, Godinho et al. 2016). The TSM colony used in this study was derived from a colony previously determined to be capable of inducing cotton plant defences (Miyazaki et al. 2013). Induced resistance of plants has previously been investigated, including on cotton seedlings and leaf discs. The controlled exposure of seedlings or leaf discs to TSM was shown to increase resistance to later inoculations of TSM measured by the development time of individual mites (Karban and Carey 1984, Miyazaki et al. 2013). If TSM in this study was inducing plant defences, this could reduce fitness in BSM and SSM when on the same plant or leaf. Previous studies have however identified BSM is a plant defence suppressor (Godinho et al. 2016), which may explain why no effects were detected between the pairing of BSM and SSM, but not why BSM cannot counter the induction of plant defences by TSM.

The second competition experiment, focusing on TSM and SSM, was conducted at a lower temperature (26 ± 1.1°C) than the first experiment and also included a second cultivar. It was evident this favoured TSM in each species pairing and cultivar, as TSM was significantly higher than each comparable SSM pairing and when alone. Between cultivars, TSM had significantly higher females alone on non-B3 than when paired with SSM on B3. There was no effect detected on the number for SSM females in each pairing and cultivar. The mean numbers of SSM were slightly lower compared with the first experiment, which may again be because of their slower development time. In contrast to Experiment 1, differences between distributions of SSM was not observed in Experiment 2.

The effect of cultivar on the mean number of female mites of both species was an unexpected result. The non-B3 cultivar averaged more female mites than the B3 cultivar. The second cultivar was included in the experiment because it was important to validate the results of the first experiment on a cultivar containing Bollgard 3 traits that is more widely grown in Australia. The activity of insecticidal proteins contained within Bollgard 3 are considered to be highly selective on lepidopteran species (Naranjo et al. 2008) and there are currently no reports that they have any toxic effect (acute or sublethal) on mites (Whitehouse et al. 2005). However, mites on transgenic maize have been shown to sequester some proteins as they are abundant in the mesophyll tissue from which they feed (Dutton et al. 2002).

The competition experiments did not find any evidence that either BSM or SSM can outcompete TSM on the same plant. There was some evidence at higher temperatures that SSM may have a higher population increase when alone. This means that if there is a changing mite species complex in Australia cotton, then it is likely due to factors other than mite species interactions. Increasing average temperatures are being observed across Australian cropping regions and the same trend around the world is projected to effect the composition of pest species (Karuppaiah and Sujayanad, 2012).

Key messages

- **There was no evidence from this study that SSM can suppress or displace TSM when present on the same cotton plant. This means if the prevalence of SSM is increasing in cotton growing regions, then it is likely due to other factors such as increasing temperatures.**
- **The increasing prevalence of SSM in crops, including cotton, may continue if the current warming trend persists.**

4.3 Field survey

Field sampling of SSM in this study detected for the first time in at least three years the presence of BSM in a cotton crop which had an established population of SSM, albeit at very low levels. The study was limited to sampling on a single occasion during the season, as no other SSM populations were reported. The field surveyed was heavily infested with SSM and supported findings from the damage experiments that the damage caused is minimal. However, these findings need to be verified with further field studies.

Key messages

- **Field sampling of SSM in this study demonstrated the mite sampling protocol is sufficient for accurately assessing SSM populations late in the season.**
- **The incidence of each species in Australian cotton should continue to be monitored so there is industry preparedness in any changes in future mite species complex, including incursions of new species.**
- **Supports findings from damage experiments reported herein that SSM populations do not warrant control even in heavily infested fields.**

4.4 Comparative life history

The life history traits of mites such as development time and fecundity strongly influence a species ability to colonise host plants. Research to date on BSM and SSM life history traits suggests that at higher temperatures, BSM has a faster development time than TSM (Silva 2002, Carey and Bradley 1982). The only other reported data of BSM on cotton had a longer development time of 17.3 days at the same temperature (25 ± 2°C) compared with 12.27 days in the present study. The slowest development occurred in SSM (13.67 days) which is in agreement with the delayed peak in the number of adult females recorded during the damage experiments, compared to TSM and BSM.

There was only one other study in the literature related to SSM, which determined the effect of mating status on the fecundity of four spider mite species (Bonato and Gutierrez 1999). There are other studies involving spider mites called strawberry spider mite, but these are not *T. lambi* (Marcano Brito et al. 1986). The lack of experimental research on SSM means this species relatively unknown from an ecological and agricultural pest perspective. The species *T. lambi* in Australia may also not be just one species and could in fact be several different species still being described as being *T. lambi* (Seeman and Beard 2011). This makes it impossible to determine whether the data from Bonato and Gutierrez (1999) relates to the SSM found to Australian cotton.

It was identified in the literature that BSM had a higher total egg count than TSM at a similar temperature (Silva 2002, Miyazaki et al. 2013). This study did not detect any difference between the rate of egg lay or total eggs per female between TSM and BSM. The total number of eggs for BSM was slightly higher at 68 ± 10 than reported by Silva (2002) at 51 ± 27 at the same temperature. The TSM total egg lay of 60 ± 5 was considerably lower than 103 ± 39 (SD) reported by Carey and Bradley (1982). Comparisons between studies are strongly influenced by host plant/cultivar, mite strain and environmental conditions, but mite response to the effects of temperature appears consistent. BSM at 30°C was reported to have a higher maximum intrinsic rate of increase (r_m) than TSM, (r_m = 0.418 and 0.364 in BSM and TSM, respectively). This was supported by Gotoh et al. (2015) who examined the role of increasing global temperatures in changing the pest status of mite species and concluded BSM to be more adapted to higher temperatures than TSM. This would suggest BSM fitness on cotton can exceed that of TSM, yet BSM has remained rare in Australian

cotton over the last three years and the cause of this remains unknown (Herron and Marshall 2019).

The sample size for SSM in this study was small and more replication is required. Due to time constraints and a delay in obtaining SSM, this study was primarily focused on collecting development time and fecundity data. It would be beneficial to collate more detailed life tables of particularly SSM at multiple temperatures to determine if a hotter climate is favouring SSM over TSM. It is important to conduct all three species in side-by-side comparisons to overcome variability noted by previous authors (Silva 2002, Bonato and Gutierrez 1999).

Key messages

- **Further optimisation of data collection is required to track numerous mites at once to improve efficiency in construction of life tables for mite species studied, including quantification of reproductive potential in SSM. Planned completion of this work in mid-2020 will facilitate publication of results.**
- **Additional life history analysis of each species at different temperatures will be important for providing more insight into their optimal thermal requirements. There are likely many other factors and interactions in the broader agricultural landscape that could be shaping the mite species complex in cotton.**

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Part 4 – Final Report Executive Summary

Three species of Tetranychus spider mites are found in Australian cotton crops. Spider mites cause damage to cotton by feeding on individual leaf cells using their chelicerae to pierce and remove the cell contents. *Tetranychus urticae* (Koch) (two-spotted spider mite: TSM) has been extensively researched in cotton and can reduce photosynthetic capacity, stomatal conductance and ultimately lead to decreased yield and fibre quality in cotton. However, no research into the damage potential or ecology of *Tetranychus ludeni* (bean spider mite: BSM) and *Tetranychus lambi* (strawberry spider mite: SSM) has been conducted. This research aimed to compare the relative damage to cotton caused by each spider mite species and to investigate spider mite ecology in cotton.

Cultures of each mite species were established in a glasshouse. Glasshouse studies were performed to compare the damage caused by each species on potted cotton plants, using a leaf damage index (LDI). TSM caused twice the level of damage than BSM and three times the damage of SSM. By week five, 33% of leaves on plants infested with TSM had defoliated. No defoliation was observed for either BSM or SSM. In addition, the damage caused by SSM was never significantly different from control plants where mites were excluded.

Laboratory studies found that average development of BSM (12.3 days) and SSM (13.7 days) from egg to adult on leaf discs was significantly slower than TSM (11.39 days). However, there was no significant difference in the mean number of eggs laid per day for TSM (6.3 eggs) and BSM (5.3 eggs). The implications of life history traits and how the result might change at higher temperatures are discussed.

Competition between mite species on single potted cotton plants was investigated in two glasshouse experiments. After two weeks the total number of females and distribution on plants was compared for each species alone or in the presence of another species. Findings from the first experiment at 30.1 ± 4.5°C suggest TSM suppressed the BSM female population and displaced SSM females from individual nodes of the plant. The second experiment at 26 ± 1.1°C included an additional cotton cultivar containing Bollgard 3 traits. Significantly higher numbers of TSM females were recorded in each pairing with SSM compared with the number of females in SSM populations alone. This was an unexpected result as TSM is considered to be declining in incidence across cotton growing regions and suggests environmental factors are contributing to the changing mite species complex.

Field surveys were conducted in northern New South Wales to determine the distribution of SSM within the cotton canopy to assess the relevance of the current mite sampling protocol for this species. Surveys indicated that the current sampling protocol would be reliable for late season sampling as mite abundance was very similar throughout the cotton canopy. The survey also detected BSM for the first time in recent years in cotton and in the same fields as an established population of SSM.

The results of this study recommend modification of current practices in cotton mite management to ensure accurate identification of the species present. This may help avoid unnecessary insecticide applications for mite species that may never reach economically damaging levels. They also suggest that there are other factors in the cotton landscape contributing to the changing mite species complex. This research provides basic research to support the sustainable management of a changing mite complex in Australian cotton.

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Appendix 1

Literature review

Introduction

Spider mites are an important agricultural pest worldwide causing crop losses and increased costs of production due to control measures (Grbić et al., 2011). The three *Tetranychus* species that occur in Australian cotton systems cause feeding damage of varying severity (Williams, 2018).

Tetranychus urticae Koch (two-spotted spider mite) was historically the main pest species while the other two species *Tetranychus lambi* Pritchard and Baker (strawberry spider mite) and *Tetranychus ludeni* Zacher (bean spider mite) seldom caused economic damage (Williams, 2018). However, in the last 10-20 years *T. urticae* has been declining in incidence and the less well researched *T. lambi* and *T. ludeni* have become more common in cotton landscapes (Herron and Wilson, 2016). Differences in damage potential between the three mite species mean that correct identification of species present is pivotal for ensuring the appropriate and justified use of insecticides (Williams, 2018).

Current management practices within the cotton industry potentially target populations of unverified species and/or infestations that may not have reached a level of economic damage (Herron and Wilson, 2016). This type of 'insurance spraying' results in increased selection pressure for resistance and potentially disrupts beneficial insect populations which in turn could lead to other pest outbreaks and additional spraying. Evaluating relative damage potential, life history traits and interspecific competitiveness of these three key species in the cotton-mite complex, as is proposed for this thesis, could lead to more targeted control in cotton and result in reduced spray applications, slower resistance evolution and a lower cost of production.

The following literature review will summarise the current state of spider mite ecology research with a particular emphasis in cotton. Methods for evaluating spider mite damage will also be examined to determine how each species of interest may be compared. The review will then examine what the current state of knowledge is of each spider mite species life history and identify any deficiencies. The interaction between spider mite species will also be assessed and whether there may be methods to detect competition between species. Finally, this review will focus on the role of integrated pest management (IPM) in cotton production and how research into insect pests contributes to an IPM strategy.

General biology, ecology and morphology of spider mites

Lifecycle

Spider mites are members of the Acari family Tetranychidae, consisting of approximately 1,200 species. They are generally less than 1mm in size and have a broad range of colours and markings (Seeman and Beard, 2011, Bolland et al., 1998). The term "spider" in the name can be attributed to the ability of Tetranychidae to produce webbing. They are plant herbivores (phytophagous) that commonly feed on leaf tissue by inserting stylet-like mouth parts to remove the cell contents (Bolland et al., 1998). The genera *Tetranychus* comprises 14 species, three of which can be found infesting cotton crops in Australia, namely *T. urticae* (TSM), *T. ludeni* (BSM) and *T. lambi* (SSM).

Generally, the females of these mite species are larger than the males. The colour and markings of these mites (see section 1.7) is based on how they commonly appear when

cotton is the host plant. Spider mites can have a variety of appearances that can be influenced by host plant and temperature even within the same species, making identification difficult, if not impractical without taxonomic expertise.

The life cycle of spider mites consists of five life stages of development (Figure 1). Eggs hatch as a larvae with three pairs of legs, followed by two nymphal stages (protonymph and deutonymph) with four pairs of legs before reaching adulthood.

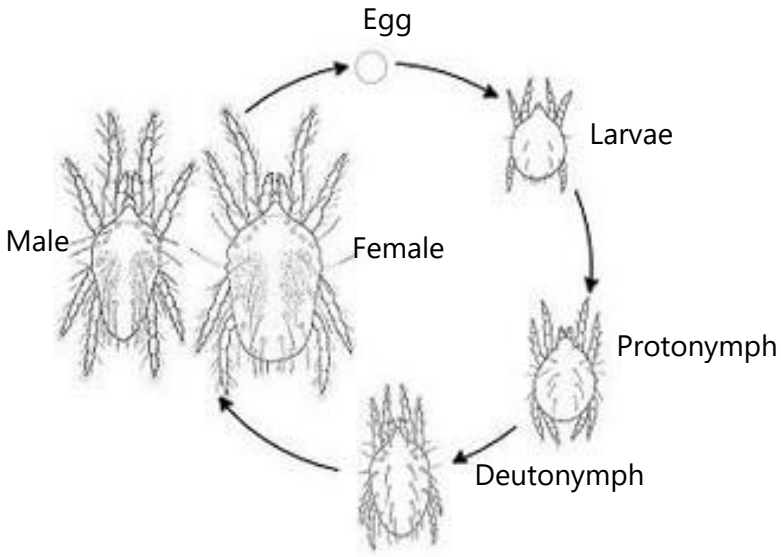

Figure 1: The life cycle of a spider mite from egg to adult (Science, March 11, 2008).

Each of these can be termed active stages which are separated by a quiescent period and a moulting phase. The males will usually develop slightly faster and stand guard alongside quiescent deutonymph females before they become adults (Carey and Bradley, 1982). This increases the likelihood of fathering offspring and supports the notion that spider mites have first insemination primacy (Helle and Sabelis, 1985). Spider mites exhibit arrhenotoky whereby females are diploid and males are haploid. This means unfertilized females can still reproduce but will only have male offspring or any F_1 females will be sterile. Fertilized females produce progeny of both sexes and typically results in a predominantly female population (Bonato and Gutierrez, 1999).

The rate of development in mites is influenced predominately by environmental conditions, particularly temperature, but also host plant quality (Wilson, 1994) and host resistance traits (Karban et al., 1987, Miyazaki et al., 2013). Compared to other pest genera of mites, *Tetranychus* spp. have been shown to mature faster and lay more eggs as temperatures increase from 24°C to 30°C (Carey and Bradley, 1982). This allows populations to increase rapidly under favourable conditions, making regular monitoring of field populations important for the timing of control decisions.

Dispersal and overwintering

All three *Tetranychus* spp. have a large host range and a wide distribution across Australian agricultural growing regions (Gutierrez and Schicha, 1983, Seeman and Beard, 2011). In the context of Australian cotton farming systems, *Tetranychus* spp. are predominately active during the warmer months where they disperse from many types of broad leaf winter weeds and volunteer or ratoon cotton to infest the new season's cotton (Wilson and Morton, 1993). Once senescence is induced at the end of the cotton season, mite populations disperse and establish on broad leaf weeds either in fallow fields, native vegetation or road sides. Dispersal may also be facilitated by the concentration of mites at

the top of a host plant forming a collective silk webbing and allowing dispersal by air currents or animals (Clotuche et al., 2011). It is likely populations are spread to a greater extent by movement of humans and vehicles/machinery across farms and even regions (Williams, 2018).

A winter diapause form can be induced by cooler temperatures and reduced photoperiod (Koveos et al., 1993). Overwintering mites do not breed and their increased tolerance for desiccation and lack of food allows mites to survive through winter (Ghazy and Suzuki, 2014). Overwintering females of TSM have a characteristic orange colouring. While it is also likely that BSM and SSM overwinter no details about induction or colour change have been reported. Wilson (1995) suggests that in temperate climates like the Namoi Valley in north western New South Wales, TSM may reside and reproduce on winter weeds. As a result populations in certain growing regions may not enter complete diapause or may do so for a short time and only in the coolest part of winter. This was supported by resistance monitoring that found incipient resistance in known resistant populations from season to season, suggestive of dilution of resistance alleles (Herron et al., 1998).

Within-plant and within-field distribution

Spider mites in a cotton crop are most commonly found on the underside of leaves, particularly where the petiole meets the leaf (basal portion) and in leaf folds (Wilson et al., 1981). These positions offer some protection from the elements and may provide a more suitable microclimate (Wilson and Morton, 1993). Surveys of populations before and after rain events show a significant decline in numbers and suggest that sheltered regions are prioritised (Reddall et al., 2007, Jeppson et al., 1975). This is particularly true for ovipositional areas to protect eggs, which are commonly placed in heavily webbed regions on the underside of leaves. Wilson and Morton (1993) found evidence of an edge effect early in the cotton season where mite populations initially established following dispersal from either adjacent crops or surrounding uncultivated vegetation. An established mite population favours main stem leaf nodes within the crop and infests the plant vertically through the vegetative phase of plant growth (Wilson et al., 1981).

Surveillance

Surveying mites within a cotton crop currently follows a visual presence/absence sampling method to determine field densities for informing control decisions based on an economic threshold and the growth stage of the crop (Williams, 2018). To develop this sampling method, TSM populations were monitored on main stem leaf nodes throughout an entire season. The highest abundance of mites was found on the main stem leaves that are three, four or five nodes below the plant terminal (Wilson et al., 1981, Wilson and Morton, 1993). From the same studies, within plant distribution and dispersal show that TSM moves up the plant as main stem nodes increase during the vegetative growth stage over the growing season. Mite density and intra-plant dispersal then declines towards the end of the season as leaf nitrogen declines and senescence begins and mite populations seek out alternative hosts (Wilson, 1995). This procedure and the accompanying economic threshold are based upon TSM infestations. Although sampling of other *Tetranychus* spp. is useful for estimating population density if identification can be made, the same level of infestation does not necessarily compare to economic thresholds of TSM because of the potential for differing levels of feeding damage severity across different mite species (Williams, 2018).

General pest history

Historically, the predominant mite pest of Australian cotton was TSM. Hence, spray thresholds and yield loss charts were developed for this species (Williams, 2018). Mite populations can usually be suppressed by mite predators (predominantly thrip species *Frankliniella occidentalis* (Pergande) and *Thrips tabaci* (Lindeman)) and late season

infestations are unlikely to cause economic loss. Hence the growth stage of the cotton crop is an important factor when making control decisions (Wilson, 1993). If natural predator populations are seasonally low or an insecticide application for another target pest reduces mite predators, the conditions may become conducive for mite populations to flare (Wilson et al., 1998). Early season mite infestations or severe mite flaring events due to reduction of natural enemies may require intervention with selective insecticides to reduce risk of economic loss (Wilson, 1993).

A key challenge in mite management is to not only conserve and utilize natural enemies to enhance pest suppression but also prevent over-reliance on chemical control that can lead to insecticide resistance. TSM has a track record for developing resistance to a range of insecticidal classes including organophosphates (Herron et al., 1998), synthetic pyrethroids (Herron et al., 2001) avermectins (Herron and Wilson, 2016) and chlorfenapyr (Herron et al., 2004). The capacity of mite species to quickly develop resistance under selection pressure is due to several biological characteristics including high fecundity, haplo-diploid sex determination (high female sex ratio) and rapid development (Gotoh et al., 2015).

Since the introduction of two-gene transgenic cotton in Australia in 2003, the reported incidence of BSM and SSM, anecdotally, increased. It is feasible that a reduction in the number of broad-spectrum sprays and improved management practices such as spraying only at threshold levels may have led to increased incidence of BSM and SSM populations in the cotton agroecosystem. Whilst SSM has been implicated in Australian cotton since at least the 1970's there is no resistance profile or experimental evidence of its damage potential (Herron et al., 1998). SSM rarely requires control, even when populations are high and therefore species identification is important for informing spray decisions (Williams, 2018). However, there is a common practice within the industry that whilst applying an insecticide for a pest such as green mirids (*Creontiades dilutes*), a prophylactic miticide is also applied (Herron and Wilson, 2016, Mckay, 2019) This would have the unintended consequence of increasing resistance selection pressure on populations of non-target or below-threshold species present in the crop.

Mite identification and host range

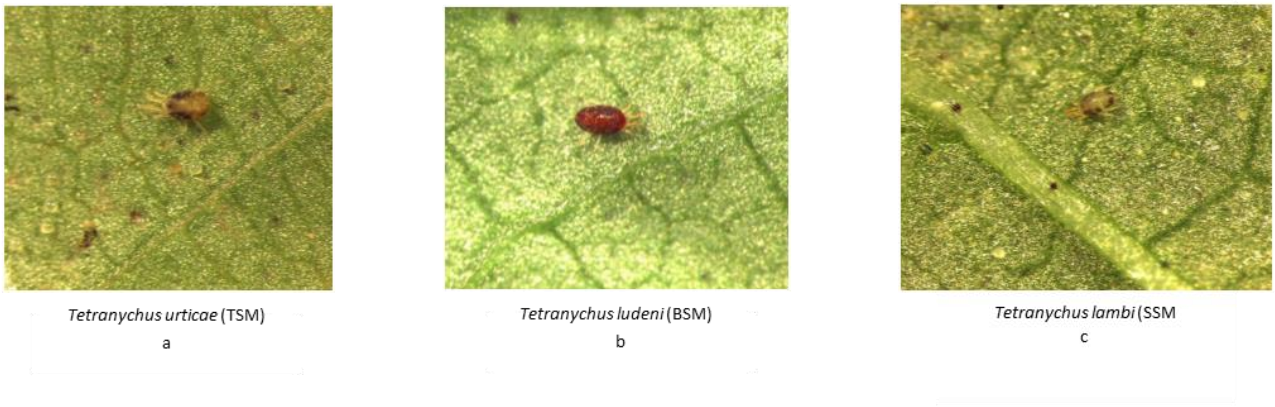

Figure 1a-c: The three *Tetranychus* spider mites found in cotton. Photos (C. Shafto, 2019)

Tetranychus urticae (TSM)

TSM is the most well-studied and the most economically important species of spider mite worldwide in crops, horticulture and ornamentals (Bolland et al., 1998). TSM is often described as a generalist herbivore because of its large host range, in excess of 870 recorded host plants, and has multiple common names such as the carmine mite or red

spider mite (Bolland et al., 1998). TSM females are approximately 0.5mm in length and can be observed with two black spots, one on either side of the abdomen but TSM can take on other colour and marking forms such as green, red or mottled spotting (Figure 2a).

Tetranychus ludeni (BSM)

This species has been identified in many parts of the world and is found across eastern Australia on a diverse range of host plants and reportedly often found in mixed populations with TSM (Gutierrez and Schicha, 1983). There are currently more than 250 recorded host plants across many different families (Beard, 2019). The females of BSM are also approximately 0.5mm in length and usually a deep red in colour, whilst males are orange or yellow in colour (Silva, 2002)(Figure 2b).

BSM is an important economic pest of vegetable crops from the *Solanum* family (Reddy, 2001) and velvet bean (*Mucuna deeringiana* Merrill) (Adango et al., 2006). BSM was first formally described in Australia in 1966 (Miller, 1966). Although initially included in the resistance monitoring schedule for cotton from 1970 onwards, resistance has not been tested for since 1980 in this species (Herron et al., 1998). A clear distinction can be made between the appearances and perhaps severity of BSM and TSM damage, but the damage potential in terms of economic loss on cotton has not been investigated. The prevalence of BSM has reportedly changed over the decades between the 1970's to the 1990's. A lower tolerance to organophosphate based insecticides from the 1970's to 1980's has been implicated as the possible cause for BSM's apparent disappearance from crops until the late 1990's (Herron et al., 1998). Current survey data of BSM prevalence across cotton growing regions report that no populations were detected between 2016 and 2019, which is in contrast to collection data from the previous decade where BSM was believed to be increasing in prevalence (Williams, 2017, Williams, 2018)

Tetranychus lambi (SSM)

SSM is the smallest of the three species and was first described in Australia in 1966 (Seeman and Beard, 2011). Elsewhere, the species has only been identified across the major Pacific Islands and Iran and has been collected on more than 60 host plants (Beard, 2019). Females measure approximately 0.3mm in length. They have a yellow straw or light green colouring with three dark green or black spots along either side of the abdomen (Figure 2c).

The males are a light yellow to translucent. The species has been identified on many introduced plants in New South Wales (Gutierrez and Schicha, 1983) and Queensland (Davis, 1968). SSM is a known pest of strawberries (Davis and Heather, 1962) and bananas (Gutierrez and Schicha, 1983). There are currently doubts whether all specimens identified as *T. lambi*, that have been documented across diverse regions in Australia, are in fact one species but might instead be multiple species yet to be described (Seeman and Beard, 2011).

It is possible SSM could have in many cases gone unnoticed prior to insect pest management becoming widely adopted by the cotton industry. SSM damage, particularly early in the crop development, can easily be missed amongst all the other biotic and abiotic damage within a cotton crop. Other factors that may have increased the reported incidence include regular crop monitoring using standardised sampling methods and increased grower and agronomist awareness of pests and beneficials. These management practices can help to identify when mites are present, but monitoring processes are currently insufficient to confidently distinguish between all mite species.

Spider mite feeding damage

The three *Tetranychus* spp. are all polyphagous plant feeders but have clearly distinct damage symptoms on cotton. Spider mites feed in every active stage of their lifecycle using highly modified chelicerae mouth parts to pierce and remove the cell contents (Bolland et al., 1998). Feeding damage by TSM reduces chlorophyll content, leading to loss of photosynthetic capacity in cotton (Reddall et al., 2007, Marcano Brito et al., 1986), cucumbers (Park and Lee, 2002), apples (Hall and Ferree, 1975) and almonds (Youngman et al., 1986).

Mite feeding behaviour causes a stepwise cascade of events initiated by mechanical injury to plant tissues by typically piercing the underside of leaves with their chelicerae. First through the lower epidermal layer into the spongy mesophyll and, in the case of adult TSM, the palisade layer resulting in a short-term increase in transpiration (Park and Lee, 2002). The plants' response is closure of stomata to control water loss. Continued feeding results in loss of chlorophyll and reduced CO2 gas exchange resulting in reduction in net photosynthesis and transpiration (Reddall et al., 2004).

Uncontrolled water loss and cell chlorosis eventually leads to partial or whole leaves dehydrating and defoliating, which is common with TSM infestations (Reddall et al., 2004). Cell chlorosis is not generally observed from BSM and SSM damage which remains as white stippling. However, the maximum damage potential has not been quantified in cotton.

Appearance of BSM and SSM damage on the upper leaf surface as white stippling can be influenced by maturity and thickness of the leaf (C Shafto, personal observation) which is supported by Park and Lee (2002) who suggested that damage could be a function of stylet length and leaf thickness. In the same study on cucumbers, the appearance of damage on the adaxial leaf surface was observed 2-3 days after injury to the abaxial surface.

Comparisons of juvenile and adult TSM damage in cucumber leaves found juveniles feeding damage extended to the spongy mesophyll whilst adult feeding reached into the palisade mesophyll (Park and Lee, 2002). Since the predominant amount of chloroplasts are found in the palisade mesophyll, adult TSM reduced chlorophyll content to a greater extent than juveniles (Park and Lee, 2002). This difference between juvenile and adult TSM damage may provide evidence to explain some of the apparent differences in feeding damage between the larger TSM and the smaller SSM.

Mite feeding damage is not limited to the cells directly fed upon as adjacent cells can also show symptoms of degenerative processes and injury (Park and Lee, 2002, Mothes and Seitz, 1982). Theories around compensatory mechanisms at the leaf and whole canopy level in cotton have been tested by Reddall et al., (2004) who hypothesised that because TSM damage in cotton was usually concentrated around the basal region of the leaf, the remaining green distal portion could compensate for the damage. However, this was not the case and reduced stomatal conductance and net photosynthesis in the distal portion was evident (Reddall et al., 2004). Undamaged leaves on infested plants also had similar relative chlorophyll content and net photosynthesis as those on undamaged and uninfested plants (Reddall et al., 2004). Evidence of compensation at the crop level does appear in advanced mite infestations where the top of the canopy becomes damaged enough to allow light penetration and regrowth from lower portions of the canopy (Reddall et al., 2007).

Reduced productivity as a result of BSM infestation has been investigated in eggplant via a screening project for resistant varieties (Reddy and Baskaran, 2006). There were clear

varietal differences in seedling mortality and total number of fruit across different levels of infestation, but further physiological effects have not been measured (Reddy and Baskaran, 2006). Similarly, the damage caused by SSM has not been quantified at either the physiological level. Hence the economic impact of this species is still largely unknown.

Quantifying mite damage

There has been a wide variety of methods used to measure mite herbivory and a few instances where multiple species have been compared. A very simple method devised by Hussey and Parr (1963) was the Leaf Damage Index (LDI). The index follows a 0–5 scoring system where each integer represents 20% of the leaf area damaged. For example, a score of 1 is equivalent to 1-20% of the scored leaf having some form of damage. Hussey and Parr (1963) were able to relate the number of adult female TSM to the LDI score. An observational scoring system such as this can be carried out in-situ and samples can be processed quickly. However, it is almost entirely reliant on the observer and scores could potentially diverge where multiple observers are used.

Similar index scales are used widely in crop disease surveys and assessment of plant genotypes for resistance (Babu et al., 2013). Tomkiewicz et al. (1993) further refined the assessment of damage caused by cassava green mite (*Mononychellus tanajoa)* by converting the LDI score of damaged leaves to a relative loss of chlorophyll content. This required measuring the chlorophyll content of damaged leaves representative of each score and measurements of total average leaf area. This resulted in a method to determine active chlorophyll tissue of a scored leaf and, if taken in-situ, allowed repeated measures over time. The calibration of the model that converts the LDI score would need to be adjusted for unrelated plants species as chlorophyll content and leaf size vary. The conversion of the LDI generates more valuable quantitative data but does require a lot more preparation and testing before its application in an experiment.

Image analysis involves processing scanned images through software such as SigmaScan™ (Inc, 2019, Kerguelen and Hoddle, 1999), Compu Eye (Bakr, 2005) or Mathematica (Wolfram Research, 2019, Škaloudová et al., 2006). Colour-defined parameters are set which discriminate between damaged and non-damaged regions. The software is then able to count the number of pixels that fall into each of the colour defined parameters and the difference can be converted to the percentage of leaf area damaged. This method removes the potential for observer effects, providing a consistent quantitative measure and has low resource requirements of a scanner and a software package able to process images. This approach has been used with TSM on leaf discs and compared with the LDI system. The results from Škaloudová et al. (2006) were that both their proposed method of the normalised proportion of leaf area damage (NPLAD) and the LDI were sensitive enough to discriminate leaf damage by different densities of adult female TSM.

Automated image analysis may have greater benefit over the LDI when processing large volumes of samples where either observer fatigue or the observer subjectivity could influence the results. These studies only looked at one species, causing damage of a consistent nature. Comparing multiple species that cause different visible damage symptoms using image analysis would likely require different sets of colour parameters to define and detect species-specific feeding damage. Image analysis, along with standard LDI scoring, is limited to measuring visible damage. This excludes physiological damage by mite feeding that could be useful in understanding the differences between mite species.

There are now widely adopted and sophisticated tools employed by agricultural research to better understand plant physiology. Marcano Brito et al. (1986) used a C^{14} dual-isotope porometer that measured photosynthesis and transpiration to compare three *Tetranychus* spp. (including TSM) in Californian cotton. All three species reduced photosynthesis and

transpiration with *Tetranychus turkestani* causing the greatest reductions at equivalent mite densities, supposedly due to a toxin produced for pre-digestion (Leigh and Hyer, 1963). The effects of TSM damage in Australian cotton has been further studied with the use of LI-COR® equipment to determine the effects of increasing mite density on net photosynthesis and photosynthetic photon flux density (PPFD). The most significant reductions in the measured parameters were in leaves from the top half of the canopy where the highest densities of mites occur. These leaves also have a higher net photosynthetic capacity than the lower older canopy leaves and so damage is more detrimental to plant growth (Reddall et al., 2007).

Since mite feeding behaviour results in a reduction of chlorophyll content, a measure of leaf chlorophyll content is an indirect method to determine a level of physiological damage after mite exposure. Studies have used wet lab techniques to extract chlorophyll content from mite effected leaves which is a simple one-time measurement of damage which could be used to compare species (Tomkiewicz et al., 1993, Park and Lee, 2002). A chlorophyll meter is a portable machine capable of measuring chlorophyll content or relative 'greenness' in-situ that can be used either for single measurements at multiple mite densities (Park and Lee, 2002) or multiple measurements over time (Reddall et al., 2004).

There are a variety of methods currently available which should allow for the accurate comparison of TSM, BSM and SSM feeding damage. Some of these methods, such as the use of LI-COR® equipment, require assess to specialized resources. Whilst others are more accessible but likely require higher numbers of replication (e.g. LDI scoring).

Quantifying Life History

Life history trait analyses are an effective means of assessing mite fitness under various conditions. A popular method of investigating insect population dynamics is to generate life tables and calculate the intrinsic rate of natural increase (Birch, 1948). Previous research has used life tables to compare host plant quality, different species and various temperatures (Wilson, 1994, Saito, 1979, Carey and Bradley, 1982). To do this, the following life history traits are required: development time from egg to adult, fecundity, generation time and adult longevity.

There are a range of factors known to influence mite development including temperature, humidity, host plant species and host plant quality. Life history traits have been used to compare the fitness of TSM on different genotypes of cotton (Miyazaki et al., 2013), to compare species or strains of mites on the same host plant under different nutritional regimes (Henneberry, 1962, Sotelo, 2014) and to compare different species of mites to inform pest status (Carey and Bradley, 1982). It has not been used however to investigate interspecific competition. The life history of TSM has been reported on red clover (*Trifolium pratense*) (Saito, 1979), various apple cultivars (Kasap, 2004), legumes (Razmjou et al., 2009) and cotton (Carey and Bradley, 1982, Miyazaki et al., 2013, Wilson, 1994).

Similar life history data has been collected from BSM on velvet bean (*Mucuna deeringiana*) (Kaimal and Ramani, 2011), black bean (*Phaseolus vulgaris L.*) (Morros and Aponte, 1994), amaranth (*Amaranthus cruentus L.*), nightshade (*Solanum macrocarpon L*) (Adango et al., 2006), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*) (Tharini et al.) and cotton (Silva, 2002). The latter study of BSM on cotton was conducted across five constant temperatures from 20°C to 30°C, with the relevant data presented in Table 2.

Only one study was identified in the literature with experimental data on SSM. The study focused on the effects of mating status on four *Tetranychus* spp. fecundity and life span, and is also included in Table 2 (Bonato and Gutierrez, 1999). Cotton was not the host plant

used to rear SSM and other important parameters such as sex ratio, intrinsic rate of increase (r_m) and mean generational time (T) were not collected.

Table 1: Life history data for TSM on cotton and BSM on cotton and on beans and SSM on beans.

Species	Host plant	Temp. °C / humidity (RH)	Photoperiod L:D	Female sex ratio %	Lifetime fecundity	Longevity of females (days)	r_m	Development time (days)	R_0
TSM (Carey and Bradley, 1982)	Cotton (*G hirsutum)* *Cultivar SJ-4.* *Cotton seedlings.*	23.8±2 Range: 50-65	16:8	67	103.30±39.30~	10.50±0.62~	0.219	19.71	74.80
		29.4±2 Range: 50-65	16:8	71	64.30±42.50~	6.10±0.44~	0.293	13.17	47.60
TSM (Miyazaki et al., 2013)	Cotton (*G hirsutum)* Sicot 71 Leaf discs.	29.5±1 RH: na	14:10	68	89.60	Not reported	0.364 (Glasshouse leaf) 0.270 (Field leaf)	11.70	71.80
BSM (Silva, 2002)	Cotton (*G hirsutum)* Leaf discs.	25 (70±10)	12:12	86	51.13±27.26*	17.38±4.84*	0.222	17.30	46.86
		30 (70±10)	12:12	83	61.26±19.46*	11.93±0.53*	0.418	9.27	48.00
BSM (Morros and Aponte, 1994)	Bean (*Phaseolus vulgaris*) Leaf discs.	26.34±3.92 (69±20)	Not reported	Not reported	118.09	18.35±9.12	0.253	19.63	77.42
SSM (Bonato and Gutierrez, 1999)	Bean (*Phaseolus vulgaris*) Leaf discs.	25±1 (70±10)	12:12	Not reported	134.5±7.20*	17.50 (median)	Not reported	Not reported	69.50±5.90^

T = mean generation time, r_m= intrinsic rate of increase, R_0= net reproductive rate. ~±SD, *±S.E, ^±SEM

The rearing methods used in such studies appear to be relatively consistent across life history studies and are done predominately under controlled laboratory conditions. Common approaches include using excised leaf discs placed on wet cotton, foam or paper towel and kept in controlled environment rooms, boxes or incubators. Leaf discs are replaced between one and four days to maintain food quality (Miyazaki et al., 2013, Saito, 1979). Carey and Bradley (1982) used whole cotton seedlings at the cotyledon stage to conduct life history experiments. However, major limitations of this study were a 20-25% loss in replicates due to immature mite mortality and the difficulty of observing mite development and egg production on whole cotton seedlings. In contrast, Wilson (1994) found that observing mites on whole cotton cotyledons to be the least suitable method for assessing TSM fecundity and development. They concluded that the use of leaf discs was a more suitable method for evaluating life history traits because mite development could be accurately observed using a stereo microscope and without the need for manipulation of the feeding substrate. In addition, by using excised leaves the mites were restricted to the cut leaf area, which increased the efficiency and accuracy of locating and recording mites and eggs.

There are many similarities amongst the life history traits, particularly between TSM and BSM and the effects of increasing temperature on a shorter T and higher r_m (Carey and Bradley, 1982, Silva, 2002). Certain life history parameters may favour an increase in BSM population over a TSM population, particularly at temperatures close to 30°C. These include a higher proportion of females in the adult cohort, shorter generation time and higher intrinsic rate of natural increase (r_m). Ultimately though, higher fecundity and net replacement rate (R_0) of TSM reported by Miyazaki et al. (2013) and Carey and Bradley (1982) suggest TSM may be at a competitive advantage on cotton due to increased reproductive capacity compared with BSM.

The sex ratio of BSM remains consistently above 80% female across four out of five temperatures tested on cotton (Silva, 2002), which is higher than TSM across the same temperature range (Carey and Bradley, 1982). There is evidence that insect and mite endosymbionts are capable of influencing a host mite or insects fecundity, mating ability, longevity and are usually passed on maternally (Enigl and Schausberger, 2007). The lack of data for SSM, particularly on cotton, makes any comparison impractical across host species as it is well observed that a host plant is a major factor in life history traits (Saito, 1979). However, there was comparable performance between BSM and SSM on the same bean species (Table 1).

Results of life-history trait analyses are influenced by the growing conditions of leaf material. It has been noted previously that both r_m and R_o values are lower on field grown material. It was suggested that field sourced leaves are smaller with higher leaf hair density and that growing conditions could affect leaf nutrition, defensive compounds and leaf thickness (Miyazaki et al., 2013). Also, the genotype of a host plant can have a significant effect as shown with certain *Gossypium* species that reduced r_m and R_0 of TSM by up to 53% (Miyazaki et al. (2013). Table 1 represents data from cotton cultivars that at each respective time period were the commonly grown cultivars and were not considered mite resistant or tolerant.

Life history traits are an important part of determining pest status and informing management strategies (Sabelis, 1991). The literature contains many examples of comparative studies in mite life history traits, many of which include TSM. A small number of these include BSM and SSM, however sometimes the life tables are incomplete. While these studies are informative, an accurate comparison between TSM, BSM and SSM is not feasible and will require life history studies to be performed concurrently on the same host

plant and under the same environmental conditions. This will enable hypotheses about displacement and competition amongst these species to be tested.

Competition between spider mites

As previously mentioned, the introduction of transgenic cotton led to a reduction in insecticide application falling from 10-14 to 0-3 sprays per season (Wilson et al., 2013). This increased the pest status of sucking insects and mites, as they were no longer being controlled as a consequence of insecticide applications for *Helicoverpa armigera*. The reduction in sprays is thought to be responsible for recent changes in species composition including resurgence in the incidence of BSM and SSM, although the former has not been detected in Australian cotton for three seasons (Herron and Marshall, 2019).

Spider mite complexes are relatively common in agricultural landscapes and interspecific competition between mite species has been investigated in various field crop and horticultural systems. In peach and apple orchards of Ontario Canada, the cohabitation and timing of infestation of *Panonychus ulmi* Koch (spring/summer) and *Tetranychus telarius* (L.) (=TSM) (summer/autumn) was studied using glasshouse and orchard trials. The glasshouse experiment results showed TSM was able to inhibit *P. ulmi* numbers regardless of the degree of initial infestation or amount of foliage on the seedling after two weeks. However, in orchard trials, *P. ulmi* reached higher numbers than that of TSM but no inhibition of the latter was evident (Foott, 1962). The species composition in this case was largely driven by seasonal changes, rather than interspecific competition in the field (Foott, 1962).

Once spider mites begin feeding on plant tissue, like any herbivore, they will usually induce a defence response from their host plant (Blaazer et al., 2018). However, *Tetranychus* spp. of mites vary in their response, even within strains of the same species. This ranges from the use of coping strategies, to actively suppressing plant defences (Godinho et al., 2016, Sarmento et al., 2011, Blaazer et al., 2018). As an indicator of plant defence, levels of proteinase inhibitors in tomato plants (*Solanum lycopersicum* L.) were increased after infestations of TSM, but were lower than control plants after an infestation of BSM or *Tetranychus evansi* (Baker and Pritchard) (Godinho et al., 2016). All the species had increased ovipositional rates on plants previously infested with BSM or *T. evansi* and lower on plants previously infested with TSM (Godinho et al., 2016). This was supported by Sarmento et al. (2011) showing the same dynamic between *T. evansi* and TSM on tomato, but included the cohabitation of the two whereby *T. evansi* would produce greater amounts of webbing in the presence of TSM, reducing TSM's ability to reach the leaf surface to feed. The cohabitation of the two also inevitably ended with the demise of TSM. It was speculated the increased webbing produced by *T. evansi* was compensating for the suppression of plant defences, which can benefit competing species (Sarmento et al., 2011).

Another case of plant defence induction may explain the ability of the Willamette spider mite (*Eotetranychus willamettei* McGregor) to reduce the population density of the economically important Pacific spider mite (*Tetranychus pacificus* McGregor) in grapevines (*Vitis vinifera*) (Karban and English-Loeb, 1990). Mite densities of either species were not negatively correlated during the season but if Willamette spider mite was released early in the season, the population density of Pacific mite was significantly reduced (English-Loeb et al., 1993). The control of Pacific mite has been repeated over multiple seasons in the field but relies on early infestation of Willamette mite on new season shoots and is believed to induce a systemic induction of plant defences (Hougen-Eitzman and Karban, 1995). A similar approach of 'vaccinating' to induce plant defenses has been used in cotton seedlings with two *Tetranychus* spp., including TSM (Karban and Carey, 1984). The total number of TSM and fecundity could be significantly reduced on seedlings previously

infested at the cotyledon stage, demonstrating a systemic induction of plant defenses in new seedling growth (Karban and Carey, 1984).

Besides the difficulties of implementing such a practice in crops, not all mite species induce plant defences (e.g. BSM) and therefore their role in interspecific competition may change depending on the mite species complex. Along with the induction of plant defences, the host plant may also release volatile compounds that attract mite predators to the plant (Blaazer et al., 2018). Whether a mite species is an inducer or suppressor may not determine the induction of these volatiles and subsequent attraction of predators (Blaazer et al., 2018). For example, *T. evansi* may possess the ability to sequester plant derived toxins into their eggs and discourage predation (Koller et al., 2007).

The occurrence of interspecific mating amongst *Tetranychus* spp. has been considered by some authors as a direct method of competition (Blaazer et al., 2018). *T. evansi,* has been documented undertaking heterospecific mating whereby males will preferentially copulate with female TSM (Sato et al., 2016). This results in those females only producing male offspring or, rarely, the production of infertile female progeny. Since spider mites exhibit first insemination primacy, any further copulation by a conspecific male is ineffective and over time this mating interference could displace another species.

However, not all reports of heterospecific mating between mite species agree that it is detrimental to one species over another (Clemente et al., 2016, Clemente et al., 2017). If the usual behaviour of a male mite is to guard the conspecific quiescent female in a bid to mate first, how frequent will heterospecific males beat the conspecific male in mating first (Clemente et al., 2017)? Since the sex ratio of *Tetranychus* spp. favours females (e.g. 65-75% female to male TSM), not all females can be guarded by a conspecific male. Regardless of the effectiveness of mating interference, *T. evansi* continues to be an invasive pest across the Mediterranean (Tsagkarakou et al., 2007) and is responsible for the displacement of TSM, mainly on non-crop hosts (Tsagkarakou et al., 2007, Ferragut et al., 2013).

Competition amongst cohabiting species can be driven directly by mating interference and web production, or indirectly by the induction of plant defences and discouraging predation (Blaazer et al., 2018). The composition of species across growing regions is likely to vary according to climatic conditions, as observed in Californian cotton (Wilson et al., 1981), cropping in Taiwan (Ho and Chen, 1993) or driven by changes in the climate overall (Gotoh et al., 2015). The latter refers to increasing average temperatures which in some cases facilitates the spread and proliferation of a pest species (Gotoh et al., 2015). Understanding the species composition in different cotton growing regions would provide information of cohabitation and help direct investigations to determine whether the BSM and SSM strains present suppress or induce plant defences as a major driver of competition and/or mating interference.

Integrated pest management for the control of mites and insecticide resistance in cotton

The Australian cotton industry adopted an integrated pest management (IPM) approach in the 1990s as part of a broader industry strategy to address concerns about selecting for insecticide resistance as well as reducing the environmental and social consequences of an industry over-reliant on broad-spectrum insecticides. The introduction of transgenic cotton producing insecticidal proteins derived from the bacterium *Bacillus thuringiensis* (Berliner), was also pivotal to this approach and resulted in a significant reduction in the use of broad-spectrum insecticides (Fitt, 2008).

The aim of using an IPM strategy is to reduce reliance on chemical insecticides and promote the use of alternative methods to manage pest populations in the broader landscape all year round while maintaining crop yield, quality, and profitability (Naranjo et al., 2008) (Figure 3). This is primarily accomplished by logical decision making about pest control using statistically robust survey protocols for pests and identification of beneficial insects, then using thresholds that account for economic loss versus the cost of control. Other strategies include eliminating weed host plants, increasing native vegetation and destruction of cotton volunteers and ratoons (Wilson et al., 2018). The improved understanding and relative importance of beneficial insects in cropping systems underpin strategies to conserve them and to encourage practices to increase their population density. This also drove industry demand for more selective insecticides for target pests and to cause minimal impact to natural enemies (Wilson et al., 2013).

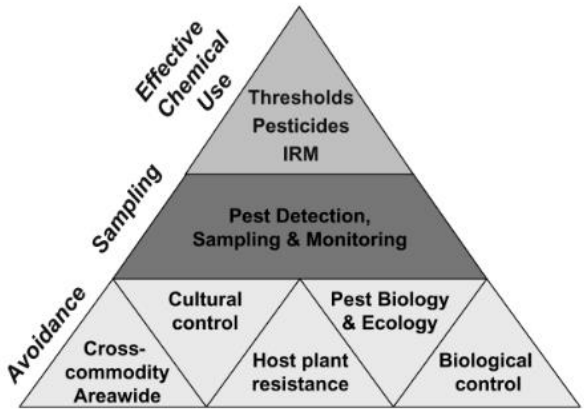

Figure 2:A ground up approach to pest management IRM=insecticide resistance monitoring (Naranjo 2001).

The sucking insect/mite complex has since become the major target for insecticidal intervention in cotton, albeit with far less economic impact than *Helicoverpa* species. When broad-spectrum insecticides like (e.g. fipronil) are used over selective options (e.g. sulfoxaflor) to control pests such as green mirid (*Creontiades dilutes*), beneficial insect populations can be negatively impacted which increases the risk of additional pest outbreaks including mites (Naranjo et al., 2008, Wilson et al., 1998). The negative impact of 'flaring' other pests is well acknowledged throughout the cotton industry. Notwithstanding recommended use of avoidance practices (Figure 4), preventative action too often relies on detection/sampling and chemical use resulting in prophylactic spraying for mites. This may not consider what species is present and does not involve an economic threshold (Herron and Wilson, 2016). Moreover, this does not align with IPM principles and does not comply with the industry endorsed Insecticide Resistance Management Strategy (IRMS) designed to reduce selection pressure.

In Australia, research conducted on early season thrip damage concluded the effects to be more cosmetic than economic and rarely affect cotton maturity significantly due to the ability of cotton plants to effectively compensate for early thrip damage (Sandras and Wilson, 1998). Thrips are an important component in early season IPM because they are extremely effective predators of mite eggs for the rest of the season, helping keep mite populations below threshold level (Wilson et al., 1996). Therefore the early season control of thrips has been discouraged to mitigate the build-up of mites later in the season (Wilson et al., 1998). This approach can be affected when controlling mirids using fipronil, which is

also active against thrips (Herron and James, 2005). The situation highlights how important an integrated approach involving regular pest sampling, using economic thresholds and careful consideration of selective insecticides is to pest management in cotton.

From the 1970's to the 1990's, cross-resistance within the organophosphate (OP) insecticidal class was clearly evident. High levels of resistance in TSM and to a lesser extent BSM populations were detected for certain OP products before commercial release, which was highly suggestive of cross-resistance to current or formerly registered products (Herron et al., 1998). OPs used against *Helicoverpa* species also increased selection pressure in mites resulting in increased resistance and reduced product efficacy (Herron et al., 1998, Herron et al., 2004). Similarly, over-reliance on the synthetic pyrethroid, bifenthrin to control *Helicoverpa* species in cotton resulted in 90% of TSM strains testing positive for pyrethroid resistance (Herron et al., 2001).

The uptake of transgenic cotton and industry adoption of more highly selective chemical control options has helped avoid scenarios of cross-resistance and lowers the selection pressure. However, resistance continues to be detected in currently registered products that are primarily or exclusively for mites. There are low levels of resistance in TSM to propargite from cotton (Herron and Marshall, 2019) and recently detected, etoxazole resistant TSM strains from New South Wales pome fruit (Herron et al., 2017). More concerning is the previously mentioned scenario of prophylactic spraying which is believed to be responsible for increased resistance to abamectin in TSM (Herron and Wilson, 2016). With the track record of TSM for resistance development and the current levels of resistance to key control products, it is clear an IRMS is invaluable to extending the useful life of these products and reducing environmental exposure to pesticides.

A better understanding of the Australian cotton mite complex in terms of damage and cohabitation can heighten grower awareness for optimising the timing of spray applications and enhancing IPM in cotton. This will increase adherence of the cotton IRMS, leading to a reduction in miticide application which could be unnecessarily increasing the selection pressure on cohabitating species or non-target mites of potential low economic importance.

Conclusion

It has been known in Australian cotton for some time that BSM and SSM are not as economically important as TSM and their management was of little consequence until their incidence, particularly SSM, became more common than TSM (Herron and Marshall, 2019). The lack of experimental evidence with regard to BSM and SSM damage has however raised questions about industry preparedness for responding to changes in the cotton-mite complex.

Further studies of BSM and SSM species ecology in terms of life history could provide more accurate information than what's available in the literature, especially for the Australian cotton context. Knowledge of their underlying life history traits would also provide the basis for exploring competition between the three species.

The changing mite species complex in Australian cotton could be influenced by interspecific interactions. Changes in cotton pest management over the last two decades has likely benefitted species such as BSM and SSM, as it has done for many other pests and beneficial insects (Wilson et al., 2018). There is substantial evidence within the literature that spider mite species are often cohabitating and the interactions between TSM, BSM and SSM has not been investigated. Further studies of comparative life-history

are needed to address knowledge gaps, particularly in relation to mite population ecology in Australian cotton.

The current mite sampling protocols are based on the field abundance and distribution of TSM. Hence, there is a need to evaluate whether the sampling technique is sufficient for detecting and accurately estimating field abundance of SSM.

The ability to accurately assess pest and beneficial populations and their impact on crop physiology is pivotal to making informed crop management decisions within an IPM framework. The IPM strategy for cotton is continually evolving and by improving the management of mite populations will optimise the cost of effectively and sustainably managing mites.