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RESEARCH ARTICLE

Field evaluation of tolerance to *Tobacco streak virus* in sunflower germplasm, and observations of seasonal disease spread

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Abstract

Strong statistical evidence was found for differences in tolerance to natural infections of *Tobacco streak virus* (TSV) in sunflower hybrids. Data from 470 plots involving 23 different sunflower hybrids tested in multiple trials over 5 years in Australia were analysed. Using a Bayesian Hierarchical Logistic Regression (BHLR) model for analysis provided: (a) a rigorous method for investigating the relative effects of hybrid, seasonal rainfall and proximity to inoculum source on the incidence of severe TSV disease; (b) a natural method for estimating the probability distributions of disease incidence in different hybrids under historical rainfall conditions; and (c) a method for undertaking all pairwise comparisons of disease incidence between hybrids while controlling the familywise error rate without any drastic reduction in statistical power. The tolerance identified in field trials was effective against the main TSV strain associated with disease outbreaks, TSV-parthenium. Glasshouse tests indicate this tolerance to also be effective against the other TSV strain found in central Queensland, TSV-crownbeard. The use of tolerant germplasm is critical to minimise the risk of TSV epidemics in sunflower in this region. We found strong statistical evidence that rainfall during the early growing months of March and April had a negative effect on the incidence of severe infection with greatly reduced disease incidence in years that had high rainfall during this period.

Introduction

Sunflower (*Helianthus annuus*) is an important oilseed crop grown in many countries in Europe, the Indian subcontinent, South and North America and Australia. The majority of the approximately 33 000 ha of production in Australia occurs in Queensland and New South Wales (Anonymous, 2014). Substantial losses due to disease caused by *Tobacco streak virus* (TSV) occurred during the mid-2000s in sunflower and mung bean crops in the central highlands region of Queensland (Sharman *et al.*, 2008, 2009). TSV has also been associated with disease epidemics in sunflower and a range of legume and vegetable crops in India, Brazil and the United States of America (Almeida *et al.*, 2005; Rabedeaux *et al.*, 2005; Kumar *et al.*, 2008). Symptoms in sunflower typically include chlorosis and distortion of leaves, severe stem and terminal necrosis, often progressing to complete collapse and death of affected plants (Kumar *et al.*, 2008; Sharman, 2015; Sharman *et al.*, 2015). The region affected by TSV spans more than 200 km from south of the town of Springsure to north of Clermont.

TSV is the type member of the genus *Ilarvirus* (family: *Bromoviridae*) which has a positive sense single-stranded RNA genome divided into three linear segments designated as RNA-1, -2 and -3 (King *et al.*, 2012). TSV is a pollen-borne virus and transmission to the leaves of susceptible hosts requires both virus-infected pollen and thrips feeding damage (Sdoodee & Teakle, 1987). The thrips species that transmit TSV in this manner

include *Frankliniella schultzei*, *Megalurothrips usitatus*, *Microcephalothrips abdominalis*, *Thrips parvispinus*, *Thrips tabaci* and *Scirtothrips dorsalis* (Klose *et al.*, 1996; Prasada Rao *et al.*, 2003; Sharman *et al.*, 2015). There are two genetically and biologically distinct TSV strains reported from central Queensland, TSV-parthenium and TSV-crownbeard (Sharman & Thomas, 2013; Sharman *et al.*, 2015). These strains were named after their respective major alternative hosts, *Parthenium hysterophorus* (parthenium) and *Verbesina encelioides* (crownbeard). While both strains naturally infect sunflower, TSV-parthenium has been the causal agent in all recent major disease outbreaks (Sharman *et al.*, 2009). Parthenium is an opportunistic, invasive weed that is established across an extensive region of central Queensland (Navie *et al.*, 1996; Adkins & Shabbir, 2014) and is an ideal host for generating TSV epidemics (Sharman *et al.*, 2009, 2015).

Control options for TSV in sunflower crops in central Queensland appear to be limited to cultural practices such as reducing the source of the virus, limiting vector populations, or the identification and use of tolerant germplasm. While some measures can be taken to control parthenium in the immediate area around crops, adequate control of parthenium across central Queensland is unlikely to be successful. At least 10 biological control agents have been released in Australia to target parthenium (Adkins & Shabbir, 2014) but it continues to infest vast regions. Effective control of the thrips vectors is also unlikely to be feasible in broad-acre farming systems as it would be uneconomical and impractical to apply the insecticides required on both the crops and surrounding parthenium-infested areas. The most effective long-term control option is likely to be the use of plant host resistance.

The limited reports of TSV resistance screening in sunflower germplasm include a trial done in India with non-replicated plots and relatively low disease pressure (Lokesh *et al.*, 2005). A subsequent trial in India was also run with non-replicated plots but TSV disease incidence was much higher and there appeared to be large differences in the tolerance of the tested hybrids (Karuna *et al.*, 2008).

In this article, our main objective was to test the hypothesis that a range of sunflower hybrids differ in their relative tolerance to natural field infections of TSV. Another objective was to characterise some aspects of disease spread such as the distance that it can spread into a crop, the association to seasonal rainfall, and if any likely benefit may be achieved with the use of barrier crops. This knowledge will be important for the development of management strategies to minimise the risk of TSV in sunflower crops.

Methods and materials

Field trial design

We conducted field trials over five consecutive seasons from 2008 to 2012 at two sites near Clermont, Queensland. Long-term weather data from Clermont (Australian bureau of meteorology site number 035019) show the prevailing wind direction throughout the day during the most common cropping period for rain-fed sunflower (February to May) is from the south-east with winds from between south to east for greater than 70% of the day. Trial sites were selected to be downwind of infestations of TSV-infected parthenium at locations where high TSV-disease levels had recently been observed in commercial sunflower crops.

The first site, hereafter referred to as Kenlogan, was approximately 49 km north of the town of Clermont and the second site, hereafter referred to as Langton Cottage, was approximately 16 km east-north-east of Clermont. We planted the field trials in late February to early March each year depending on suitable rain events for planting. In 2008, only the Kenlogan trial site was used while both sites were used in subsequent years.

Field observations in commercial crops indicated an edge effect which resulted in a higher incidence of TSV-affected plants close to the paddock boundary and downwind of the areas harbouring TSV-infected parthenium. To minimise any effect, trial sites had a long narrow, randomised block design parallel to the edge of the cropping area, downwind of areas infested with parthenium and no plot further than 20 m from the edge. A planting density of 35 000 plants per hectare was used as is recommended for commercial sunflower production in the rain-fed area of central Queensland.

The design of the trials was altered over the 5-year period to reflect the needs of the industry and to test new hybrids. In the 2008 and 2009 trials, the plot size was two rows at 1 m apart by 8 m long, containing about 55 plants and replicated four times in a randomised block design. The same design was used in 2010 with additional larger plots for three hybrids; two hybrids with good tolerance to TSV (Hysun 304 and NH2201) and one with poor tolerance (Ausigold 61). These larger plots were six rows by 16 m, containing about 340 plants and replicated three times. In 2011 and 2012, hybrids were tested in plot sizes of four rows by 16 m, containing about 225 plants and replicated six times. The hybrids tested in each trial are shown in Fig. 1.

Rating for TSV disease

We rated sunflower plants for severe TSV disease symptoms that would prevent harvesting. For the field trials,

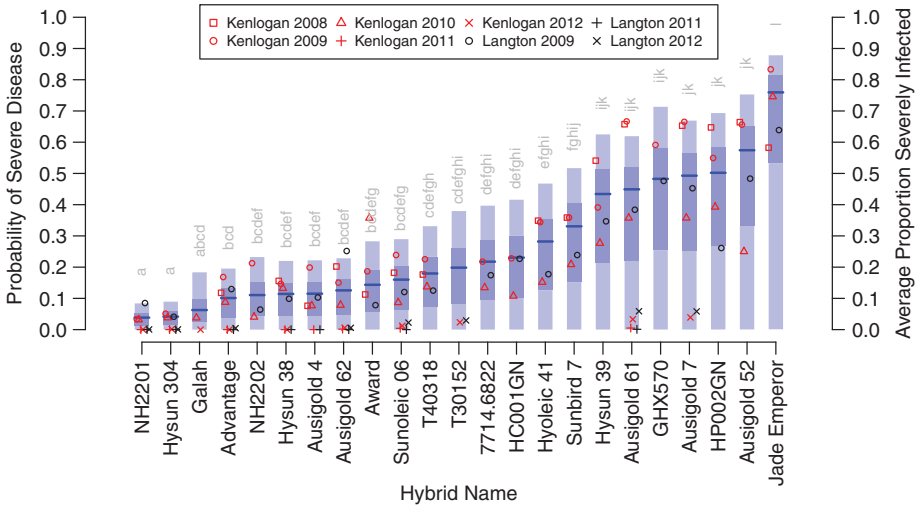

Figure 1 Modelled and observed probabilities of disease incidence for different hybrids. Horizontal dark blue lines show the modelled median, dark coloured, inner rectangles span the 25th–75th percentiles, and outer rectangles span the 95% predictive interval. The position of symbols shows the average proportion of severely diseased plants for each hybrid for each site/year combination. Red symbols represent data for the Kenlogan site and black symbols for Langton Cottage. Grey letters above the blue rectangles show the groupings from Table 2.

we assessed plants at 2 months post planting, rating for severe TSV symptoms including death, stem necrosis leading to lodging and heads severely reduced in size (Fig. 2). Disease incidence was considered to be the proportion of total plants that were severely infected from each trial plot or disease count from commercial crops. To confirm TSV infections and the strain present from representative samples, TSV ELISA and RNA 3 strain-specific PCR were done as previously described (Sharman *et al.*, 2009; Sharman & Thomas, 2013).

Statistical analysis

The data we collected consisted of counts of disease incidence in plots to which different sunflower hybrids had been randomly assigned. Such data are most naturally modelled as arising as samples from a binomial distribution with parameters P (the probability of disease) and n (the total number of plants counted in each plot). The probability of disease in each plot is modelled as a function of a number of contributing factors, such as the sunflower hybrid, the amount of rainfall and the proximity to the virus source, parthenium weed. An appropriate statistical model for modelling these probabilities (that take values between 0 and 1) is a logistic regression model.

Such a logistic regression could have been performed as a standard Generalised Linear Model or Generalised Linear Mixed Model, however, we sought to test if a Bayesian Hierarchical Logistic Regression (BHLR) model offered a more robust means of analysis. In order to address our research questions we sought to determine: (a) what the expected probability distributions of disease for different hybrids under historical rainfall conditions were and (b) how does disease resistance compare between each of the 23 hybrids.

We aimed to model the probability of sunflower hybrids having severe TSV disease and the effect on this probability from variables including the hybrid, rainfall in March or April and distance from the source of inoculum (parthenium weed). Total rainfalls for the months of March and April were included in the analysis because this is the time period that most commercial crops in central Queensland are germinating and are most susceptible to TSV infection. Field observations indicated that variation in rainfall during this early crop stage had a marked effect on TSV disease. The BHLR model development is described in Appendix S1 (Supporting Information) or from the corresponding author.

Movement of TSV into crops and effect of barrier crops

To assess potential edge effects and the distance that TSV disease can typically move into commercial crops, we visually estimated severe TSV disease incidence from at least 300 plants at different distances from the edge of crops. Disease counts were done along transect lines perpendicular to an edge of the crop which was downwind of a parthenium-infested area. Disease counts were tested by chi-square to test the null hypothesis that disease incidence did not change with distance from the edge of the crop.

As part of the 2009 and 2010 trials at the Kenlogan site, we included additional treatments to test the effect of a fast growing barrier crop. Test plots of a TSV-susceptible

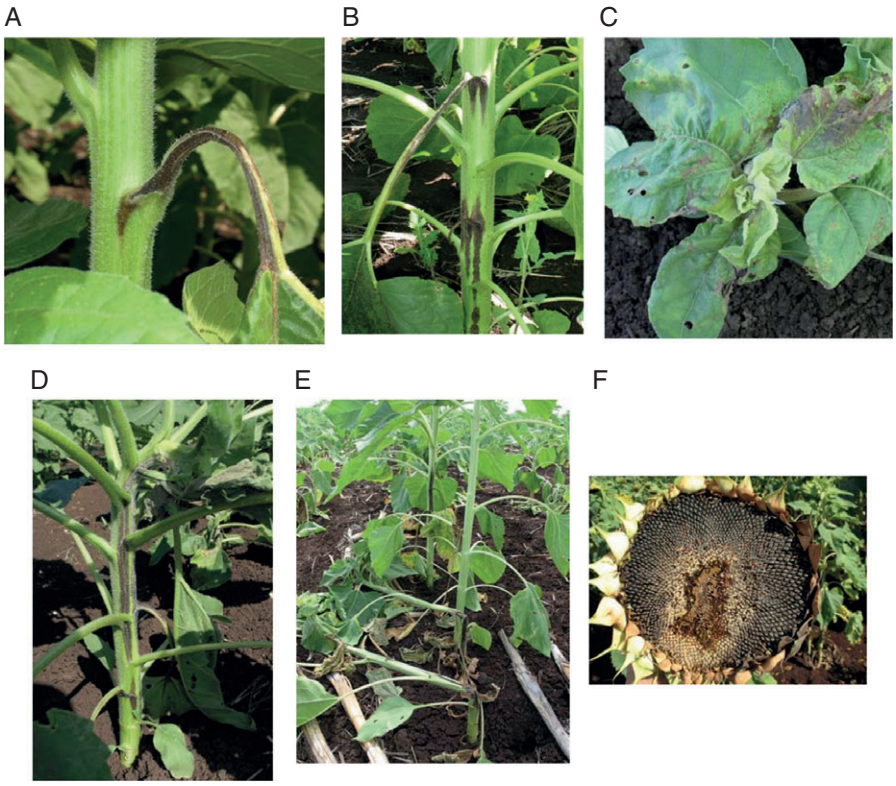

Figure 2 The range of typical *Tobacco streak virus* symptoms observed in sunflower from the field trials. Mild symptoms commonly seen but not rated as 'severe' in the field trials: (A) mild symptoms of isolated necrotic lesions on petioles; (B) necrotic lesions on petioles with spreading necrosis on the stem but not causing plant death or collapse. Severe symptoms that prevented harvesting of marketable seed included: (C) severe chlorosis and necrosis on young plants leading to death; (D) severe stem and terminal necrosis and death; (E) severe stem necrosis resulting in lodging; and (F) severe distortion of mature flower head and seeds.

hybrid (Ausigold 61) were grown within a block of forage sorghum which was planted at the same time as the sunflower and with 8 m of sorghum between the sunflower test plots and the edge of the crop. Severe TSV disease incidence was estimated visually and by diagnostic assays for representative samples as described above in the section for rating for TSV disease.

Comparison of sunflower hybrid reactions with TSV-parthenium and TSV-crownbeard strains

Reference cultures of TSV-parthenium isolate-1973 and TSV-crownbeard isolate-2334 were maintained as previously described (Sharman & Thomas, 2013). To compare the reactions of these distinct TSV strains on sunflower hybrids, they were manually inoculated onto test plants of hybrids with good tolerance (Hysun 304 and NH2201) and poor tolerance (Ausigold 61) to TSV-parthenium as determined in the field trials. Manual inoculations were done using 0.1 M phosphate buffer with sodium sulphite added and a mix of diatomaceous earth and carborundum as abrasives. Test plants were rated visually for severe disease and tested by TSV ELISA as described above at 8 days post inoculation.

Results

A range of TSV symptoms were observed in naturally infected sunflowers from field trials and commercial crops. While only severe symptoms that would prevent harvesting were considered for the assessment of tolerance, symptoms also included small necrotic lesions on petioles, through to severe stem and terminal necrosis, complete plant collapse and death (Fig. 2).

Field trials for TSV tolerance in sunflower hybrids

Over the course of the 5 year study, the proportion of total plants that were severely infected from each plot was determined from a total of 470 plots within the randomised block designs. The overall observed incidences of severe disease for each hybrid in each year/site combination along with the predicted probability distributions of

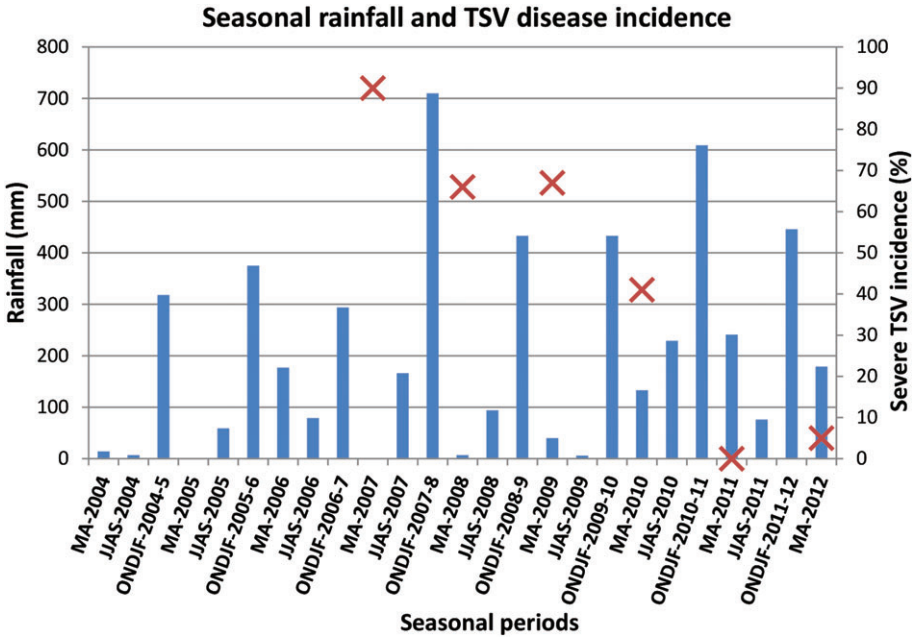

Figure 3 The relationship between changes in seasonal rainfall and incidence of severe *Tobacco streak virus* (TSV) disease in susceptible sunflower hybrid Ausigold 61 at the Kenlogan site. Accumulated rainfall data (vertical bars) and severe TSV disease incidence (red crosses) are shown. Sunflower crops are planted in late February to early March. Seasonal periods are March to April (MA) when crops are young and most susceptible to TSV infection. The low rainfall cooler months are June to September (JJAS) and the higher rainfall months across summer are October to February (ONDJF).

severe disease under historical rainfall distributions using the BHLR model are shown in Fig. 1.

The BHLR analysis of the field trial results demonstrated that all hybrids, with the exception of Jade Emperor showed statistical evidence that the hybrid-specific intercepts were non-zero (i.e. 95% credible intervals for the hybrid-specific intercepts did not enclose zero; Table S1). One way to think about the hybrid-specific intercept term in our model is that a value of zero (in the absence of other factors) corresponds to a 50% probability of a plant showing severe disease symptoms. Therefore, all hybrids except for Jade Emperor showed evidence of disease incidence of below 50% in the absence of other factors. Table S1 provides the summary of results from the 253 pairwise comparisons of each hybrid and this is also illustrated in Fig. 1. The 23 hybrids tested fell into 12 different susceptibility groupings (labelled with letters a–l; Fig. 1) in terms of the credible intervals of their differences.

Disease incidence varied greatly between seasons and our observations indicate this was closely associated with rainfall. As a comparison of relative disease incidence over the 5-year period, severe TSV disease incidence in the susceptible hybrid Ausigold 61 at the Kenlogan site was 90% in 2007, 66% in 2008, 67% in 2009, 41% in 2010, 0.5% in 2011 and 4.8% in 2012 (Fig. 3).

Table 1 Means and 95% credible intervals for parameters other than hybrid-specific intercepts in the BHLR model

Parameter (descriptor)[a]	Mean (2.5th percentile, 97.5th percentile)[b]
μ (mean for hybrid effects)	−2.13 (−2.71, −1.55)
σ_0 (std. dev. for hybrid effects)	1.20 (0.89, 1.71)
β_1 (coefficient for March rainfall)	−1.10 (−1.21, −0.99)
β_2 (coefficient for April rainfall)	−1.47 (−1.95, −1.02)
β_3 (coefficient for rainfall interaction)	−1.32 (−1.78, −0.89)
β_4 (coefficient for proximity)	−2.13E-2 (−8.77E-2, 4.22E-2)
σ_ε (std. dev. for residual variation in plots)	0.89 (0.81, 0.98)

[a]The parameters shown are those appearing in Equation (2) described in Appendix S1.
[b]The estimated mean and 95% credible intervals for the parameters.

The effects for rainfall in March, April and their interaction all showed strong statistical evidence of being non-zero (95% credible intervals did not contain zero; Table 1) and negative, indicating that when rainfall was high in March or April, the probability of observing severe disease decreased. This supports our observations that the worst TSV epidemics were observed when plantings that occurred between late February to early March were preceded by summer rains and followed by dry conditions throughout March-April. This period is when plants were young and most susceptible to infection and

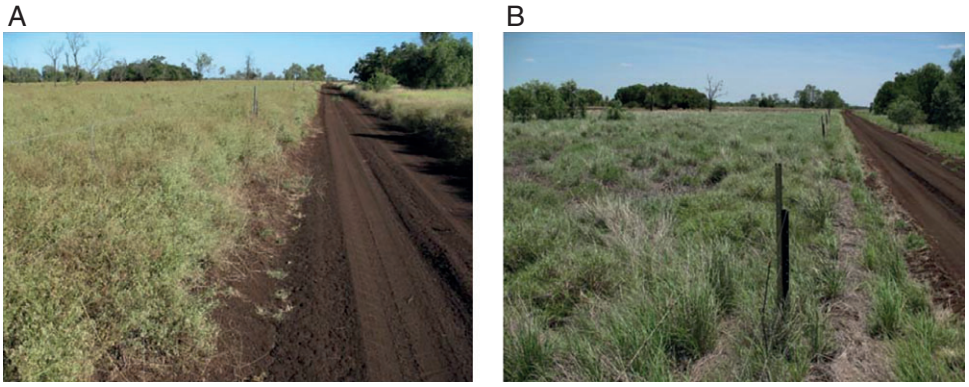

Figure 4 Photos of the same site north of Clermont, taken in April 2008 (A) and February 2014 (B) illustrating the dramatic change in the parthenium population over the 6-year period.

conditions were favourable for high thrips populations which enabled damaging TSV epidemics to develop. For completeness, Table 1 also includes the means and credible intervals for the hierarchical parameters (μ and σ_0) over hybrid effects and the residual error variance.

Fig. 1 shows the modelled median incidence of severe disease for each sunflower hybrid and 2.5th, 25th, 75th and 97.5th percentiles of the posterior predictive distributions in the absence of residual variation within plots. These intervals can be interpreted as the expected distributions (range) of disease incidence under historic rainfall conditions. The median and the widths of the intervals in Fig. 1 are largely controlled by different sunflower hybrids.

Parthenium is the major source of TSV in central Queensland (Sharman *et al.*, 2009). This invasive weed proliferates in disturbed areas and is suppressed by dense ground cover (Adkins & Shabbir, 2014). Fig. 4 illustrates a dramatic change from an almost pure stand of parthenium in 2008 after several years of El Niño drought conditions (data not shown), to an almost pure stand of perennial grasses in 2014 after several favourable years of rain during which grasses competed effectively with parthenium across much of central Queensland. This resulted in reduced inoculum source (TSV-infected parthenium) and thrips populations across most of central Queensland and in areas adjacent to the field trial sites.

Historical observations from the nearest weather station (Australian bureau of meteorology station number 035094) for the period of 1963–2014 were used to predict the expected frequency of severe TSV outbreaks. It could be expected that conditions with equal or lower rainfall than those observed in 2008 and 2009, for the months most important to sunflower crop establishment, March and April (as illustrated in Fig. 3), have a probability of occurrence of 11% and would therefore occur on average roughly 1 year in 10.

Table 2 Tobacco streak virus (TSV) disease incidence in commercial crop of sunflower hybrid Sunbird 7 to assess edge effect and distance of movement into crop

5 weeks [a]	8 weeks
5 m, 109/321 (25%) [b]	5 m, 168/286 (37%)
50 m, 36/421 (8%)	60 m, 135/250 (35%)
150 m, 23/286 (7%)	150 m, 90/257 (26%)
350 m, 22/395 (6%)	300 m, 67/266 (20%)
400 m, 30/622 (5%)	
$\chi^2 = 152.2$; $P < 0.001$ [c]	$\chi^2 = 33.3$; $P < 0.001$

[a]Age post planting of sunflower hybrid Sunbird 7. Transects ran in the direction of prevailing wind, from SE to NW, perpendicular to crop edge.
[b]Distance from edge of crop, number of plants with severe TSV symptoms/number of symptomless plants and corresponding percent incidence of severe TSV infection.
[c]Chi-square (χ^2) value for comparison frequency of symptomatic and symptomless plants with significance level indicated.

Movement of TSV into crops and effect of barrier crops

The incidence of TSV disease appeared to be reduced with the use of an 8 m wide barrier crop of fast growing forage sorghum. In the 2009 Kenlogan trial, the plot of the TSV-susceptible hybrid, Ausigold 61 within the sorghum barrier crop had an incidence of 33% severe TSV infection compared with 77% for a comparable plot the same distance from the crop edge but surrounded by sunflower. The effect was less pronounced in the 2010 Kenlogan trial for the same sunflower hybrid with 27% severe TSV incidence within the barrier crop compared with 35% outside the barrier.

In a commercial crop of hybrid Sunbird 7 grown adjacent to the Langton Cottage trial site in 2008, a strong edge effect was clearly demonstrated by the

significantly higher incidence of severely infected sunflower plants closer to the edge of the crop which was located downwind of a weedy area infested with TSV-infected parthenium (Table 2). This effect was more pronounced at 5 weeks post planting but was still significant at 8 weeks post planting. The effect of prevailing wind direction was further demonstrated from a second paddock of the same hybrid, planted at the same time and located upwind of the parthenium-infested area. At the 5 week disease count, TSV-disease incidence was 3% at 5 m upwind of the weedy area, compared with 25% at 5 m downwind of the same area (data not shown in Table 2).

At another location close to the Langton Cottage trial site in 2009, well within the boundary of a crop of sunflower hybrid Sunbird 7 and approximately 1.2 km downwind of the nearest source of TSV-infected parthenium, severe disease incidence ranged from 16 to 22% from four counts. This crop was not flowering so all TSV inoculum would have been from outside the crop.

These field observations are supported by evidence from the BHLR analysis of the 2008–2012 field trial data from Kenlogan and Langton Cottage with the coefficient for distance from parthenium having an 82% probability of taking a negative value, providing some evidence that increasing distance from parthenium reduced disease incidence across the relatively narrow width (less than 20 m) of the field trials. However, we did not consider there to be strong evidence that this term had an effect, as the 95% credible interval enveloped the value zero (β_4, coefficient for proximity; Table 1).

Comparison of sunflower hybrid reactions with TSV-parthenium and TSV-crownbeard strains

There were only minor differences between the observed symptoms of TSV-parthenium and TSV-crownbeard on the sunflower hybrids NH2201, Hysun 304 and Ausigold 61 tested in the glasshouse (Table 3). However, during field surveys over several years, natural infections of TSV-crownbeard in commercial sunflower crops appeared to cause a less severe disease than was commonly the case with TSV-parthenium and was only observed in a few locations.

In the field trials, TSV-parthenium was the only strain present and sunflower hybrids NH2201 and Hysun 304 displayed good tolerance against natural infections while there was strong statistical evidence that sunflower hybrid Ausigold 61 had higher incidence of TSV disease (Fig. 1). For example, from the Kenlogan trial in 2009, the severe disease incidence for hybrids NH2201 and Hysun 304 was 3–5% while for hybrid Ausigold 61 it was 67%.

Glasshouse observations indicated that manual inoculation was more severe than field testing and masked some differences that were apparent with field screening. Although the differences were less pronounced compared with the field trials, this pattern of good and poor tolerance was also observed for both TSV strains in the glasshouse testing. The susceptible control plants, mung beans (*Vigna radiata*) and French beans (*Phaseolus vulgaris*), were 100% infected (Table 3).

Table 3 Glasshouse comparison of reaction of TSV-parthenium and TSV-crownbeard strains on sunflower hybrids and susceptible hosts

Test host	TSV-parthenium (isolate-1973)	TSV-crownbeard (isolate-2334)
Sunflower, Ausigold 61 [a]	15/15 (100%) [b]	7/8 (88%)
Sunflower, NH2201	8/15 (53%)	4/10 (40%)
Sunflower, Hysun 304	10/15 (67%)	2/9 (22%)
Vigna radiata	6/6 (100%)	6/6 (100%)
Phaseolus vulgaris	5/5 (100%)	5/5 (100%)

[a]As determined from field trials (Fig. 1), sunflower hybrid Ausigold 61 was susceptible to severe TSV infection while hybrids NH2201 and Hysun 304 had good tolerance.
[b]Number of severely diseased plants out of total tested and percent infected.

Discussion

This is the first detailed report of the relative tolerance to TSV for a range of sunflower hybrids in Australia. We found strong statistical evidence that several hybrids displayed better tolerance to field infections of TSV compared with susceptible hybrids in multiple trials over several years (Fig. 1). Notably, hybrids NH2201, Hysun 304, Galah, Advantage, NH2202, Hysun 38, Ausigold 4, Ausigold 62, Award and Sunoleic 6 listed in groupings a and b (Fig. 1) performed very well even in years with high TSV disease pressure. We did not attempt to identify the genetic basis of the observed tolerance to TSV in sunflower hybrids. However, the high levels of tolerance in a few hybrids suggest that this may be a fruitful area for further research.

We previously tested 33 individual plants from 23 species from the Clermont area from within, or close to the trial sites by strain-specific PCRs and found all were infected with TSV-parthenium strain (Sharman *et al.*, 2015). While TSV-crownbeard has a wide host range, we only ever found TSV-crownbeard in locations where crownbeard grew. Our testing of representative samples from the trial sites and the fact that both trial sites were at least 50 km from the nearest known locations of crownbeard, provided strong evidence that TSV-parthenium was the only TSV strain present at the field trials during our study.

Our glasshouse screening results indicate that the observed field tolerance to TSV-parthenium is likely to also confer tolerance to the distinct strain,

TSV-crownbeard which appears to induce a less severe disease and is relatively uncommon in sunflower crops compared with TSV-parthenium. Given these two TSV strains are genetically and biologically distinct (Sharman *et al.*, 2015), the tolerances we have identified in sunflower hybrids may also be effective in other regions affected by TSV such as India.

In this study, the use of a BHLR model has provided an elegant method by which we could: (a) answer the same questions that can be answered using traditional frequentist approaches; (b) quantify the probability distributions of severe disease incidence under historical rainfall conditions using samples from the posterior distribution; and (c) undertake a large number of pairwise comparisons (253 in total) without being overly concerned about controlling familywise error rates. We should see greater uptake of these methods in the future with advances in the availability of statistical software for fitting complex BHLR models, like *Stan*, the recently developed probabilistic programming language by the Stan Development Team (2014) used in this study. To aid this, we have made the data and Stan programming code used in this analysis available from the corresponding author.

Bayesian statistical methods can provide an advantage over traditional frequentist methods in addressing the questions in this study. Firstly, samples from the posterior distribution and from the historical rainfall distribution allowed us to easily combine the knowledge gained from the field experiment with new data (i.e. the historical data; external to the experiment) to make some predictions about expected future levels of disease incidence. Secondly, Bayesian hierarchical models achieve a natural 'shrinkage' of the effects of hybrids towards each other that implicitly makes all pairwise comparisons conservative. This simplifies the process of undertaking multiple comparisons and has recently been argued as yielding more efficient estimates than under the traditional frequentist approach of applying corrections to *post-hoc* analyses (Gelman *et al.*, 2012).

For the reasons outlined above, we chose to use the Bayesian statistical procedure, a BHLR rather than a frequentist method such as a Generalised Linear Model. This approach is similar to that presented by Zeger & Karim (1991), but makes use of the Hamiltonian Monte Carlo (Betancourt & Girolami, 2015) rather than the more traditional Gibbs sampler to sample from the posterior distribution.

The high level of variation in disease incidence between years illustrates the importance of conducting these replicated trials over several seasons and different sites to ensure a rigorous assessment of field tolerance to TSV. Given the widespread occurrence of TSV in the parthenium population in central Queensland (Sharman *et al.*, 2009), we expect TSV inoculum from surrounding parthenium will pose a risk to sunflower crops for the foreseeable future. It will therefore be important to monitor the relative tolerance to TSV for any new hybrids and we hope that the new information presented in this study may be utilised by breeders to predict the tolerance of related hybrids.

Screening hybrid tolerance to TSV by manual inoculation in glasshouse conditions appeared to be too severe and was not a reliable method to identify differences in tolerance observed in the field trials. While manual inoculation may be useful for comparisons of very different levels of tolerance, testing by field trials was a better method to accurately assess the true tolerance of hybrids under natural conditions. Other techniques have been reported for inoculation of very young sunflower seedlings by injuring the growing point (Sundaresha *et al.*, 2012) which may warrant further comparison with standard manual inoculation. While not as easy as manual inoculation, the use of thrips and TSV-infected pollen for transmission tests in caged experiments may be a better approximation of tolerance screening under field conditions.

The severity of a TSV disease epidemic is determined by a complex interaction of factors including the growth stages of the virus source (parthenium) and the susceptible crop, the size and dispersal behaviour of the thrips population feeding on the virus source, and the orientation of the crop in relation to prevailing winds. We observed strong statistical evidence of lower incidences of TSV during periods of regular rainfall in the critical growing months of March and April, most likely as a result of reduced total inoculum and thrips populations. Almeida & Corso (1991) also observed a correlation between increased accumulated rainfall and a marked decrease in thrips populations and correspondingly lower levels of TSV disease incidence in soybean in Brazil. Lokesh *et al.* (2005) observed very low TSV disease incidence related to high rainfall which was unfavourable for vector populations.

Rainfall in central Queensland is often sporadic, making it difficult to predict the dry periods which increase the risk of TSV disease. The most common time of year for planting rain-fed sunflowers is late summer to early autumn (February to March) which also coincides with the main growing periods of parthenium. Where irrigation is available, the risk of TSV can be greatly reduced when crops are planted in Spring (September–November) when there is generally much less flowering parthenium. Similar strategies of changing planting times to avoid peak influx of TSV inoculum have also been recommended for soybean in Brazil (Almeida & Corso, 1991) and sunflower in India (Shirshikar, 2003).

Unfortunately, irrigated areas are limited in central Queensland and this strategy would be unavailable to most growers.

To minimise the risk of TSV disease in sunflower crops in central Queensland several approaches are recommended. Avoid planting downwind of large areas of flowering parthenium which is the major source of TSV that moves into crops (Sharman *et al.*, 2009). The use of a barrier crop such as sorghum, or some means of spatial separation of the crop from flowering parthenium may help to reduce the risk of severe damage near the edge of crops but is unlikely to restrict the long distance dispersal of thrips carrying TSV-infected pollen into crops. Slashing or herbicide control of surrounding parthenium prior to planting susceptible crops is also advisable to reduce TSV inoculum during the most susceptible early crop stage. The use of tolerant hybrids identified in this study will greatly reduce the risk of significant losses due to TSV in central Queensland and potentially other regions around the world where TSV affects sunflowers.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Means and 95% credible intervals for parameters in the BHLR model. Appendix S1. BHLR development.