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Towards area wide management of insect vectored viruses of tomatoes in the Bowen district

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ABSTRACT

The Bowen region of Northern Queensland is an important winter production area for tomatoes in Australia. There are three economically important viruses in the region that affect tomato, *Tomato yellow leaf curl virus* (TYLCV), *Tomato spotted wilt virus* (TSWV) and *Potato leafroll virus* (PLRV), which are vectored by whiteflies, thrips and aphids, respectively. An area wide management approach is required to lower the primary inoculum throughout the district. To this end, we undertook investigations into the virus incidence and alternative hosts for the virus and vectors in different cropping regions throughout the district, as well as local management options such as insecticide application and possible non-host cover crops for the wet-season break in production. The initial incidence of *Potato leafroll virus* was very high, most probably due to abnormal weather patterns for the district, and has ceased to be a problem. *Tomato yellow leaf curl virus* is a continual problem even at the beginning of the season, indicating large reservoir host(s) in the environment. Only four alternative hosts have been identified: *Stachytarpheta jamaicensis* (TSWV), *Solanum americanum* (PLRV and TYLCV) *Trianthema portulacastrum* (TYLCV), and *Amaranthus viridis*(TLYCV). Different insecticide and application options were trialled for protection against *Tomato yellow leaf curl virus*, with the best possible option yielding marketable fruit more than ninety percent of a resistant hybrid. A trial of yield vs time of infection of TYLCV found that whitefly exclusion for 6 weeks post-transplant yielded an average increase of nearly three kilograms of marketable fruit per plant. A number of pulse crops have been confirmed as non-hosts of tomato yellow leaf curl for use as cover crops in the wet-season break. Most of the production has moved to dual resistant TYLCV/TSWV hybrids, though an area wide management program still needs to be established to reduce the primary inoculum throughout the district, giving growers more varietal options, especially early in the season.

1. Introduction

The Bowen region (Queensland, Australia; −20.0074, 148.2433) is a strategic window of winter production of tomatoes in Australia, with a farm gate value of AUD$165 million (2013) per year. There are currently three economically important viruses in the region that affect tomato, the whitefly-transmitted begomovirus *Tomato yellow leaf curl virus* (TYLCV), the thrips-transmitted tospovirus *Tomato spotted wilt virus* (TSWV), and the aphid-transmitted polerovirus *Potato leafroll virus* (PLRV). TYLCV has only been present in the district since 2011 (S. van Brunschot pers. Comm.) and this presented an opportunity to monitor the establishment and spread of the virus in a new area. Both TSWV and PLRV have been established in the region for a number of years with sporadic, but significant outbreaks. Importantly for virus epidemiology, there is a break in production during the summer wet-season (November-March) that helps to reduce local inoculum levels.

Area wide management approaches are an effective way to control diseases with airborne vectors, and are a component of integrated disease management principles. The main goal of this management strategy is the general lowering of the primary viral inoculum levels throughout the district, thus lowering the incidence and severity of outbreaks and delaying epidemics. Bassanezi et al. (2012) show that, for citrus greening, an area wide approach is more effective than local management for controlling disease progression. The establishment of an area wide management protocol requires investigation into the local specificities of the ecology of the viruses and vectors. A central idea to

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this is the determination of reservoir hosts in the district that provide the bridge for the viruses/vectors over the wet season break in production. *Tomato yellow leaf curl virus* has a number of identified hosts belonging to 18 different families (Kil et al., 2014; Papayiannis et al., 2010). *Tomato spotted wilt virus* has an extensive host range, with new host species being frequently reported. A review in 1987 describes 230 recognised host species (Cho et al., 1987), and a more updated list 941 species from 85 families as having natural infections with TSWV (Parrella et al., 2003). A number of the proposed hosts of TSWV could be attributed to other Tospoviruses, as the bulk of work was done previous to molecular techniques. Hosts for PLRV have been identified, mainly in *Solanaceae*, but have also been found in seven other families (Natti et al., 1953; Thomas, 1993; Thomas and Hassan, 2002). With the broad range of families hosting these viruses, viral reservoir host discovery in the Bowen region should be conducted in all of the major broadleaf weeds, not just Solanaceous species.

Local management options have the ability to both lower primary inoculum levels and the secondary spread throughout the crop. Local primary inoculum levels can be reduced through the use of non-host cover crops, limiting the number of host weeds present in fallow fields over the production break (Jones, 2004). This simple crop rotation would possibly increase grower income, and limit the green bridges of volunteer plants present in the cropping area as hosts for the virus and vectors. Slowing the in-crop transmission of the virus can have a protective effect to maintaining crop yields. The use of pesticides, especially systemic insecticides, is a standard response for pest control and may be useful to limit the secondary spread of disease, especially for persistently transmitted viruses like TYLCV (Polston and Anderson, 1997). Local management may be the only option if you cannot reduce the primary inoculum source, due to large numbers and wide distribution of reservoir hosts of the viruses. In the case of TYLCV, Macedo et al., 2017Macedo et al. (2017) found that continuous primary infections are the most important factor in the spread of TYLCV in tomato crops. We have investigated the ability to increase yield through different application methods of insecticide with a TYLCV susceptible variety under high virus pressure.

This study was to increase the knowledge of the interaction of the hosts, vectors and viruses in the region to form a basis to inform growers of the options to better integrate management throughout the region.

2. Materials and methods

2.1. Surveys

2.1.1. Commercial tomato disease surveys

Surveys of commercial tomato crops were conducted in the Bowen district (Queensland, Australia) during the end of the 2012 season. All three major production areas within the district were surveyed. These production areas are comprised of properties along Euri Creek Rd, Collinsville Rd and within the Delta area. Fig. 1 shows the spatial distances among these areas and the 2012 survey sites. The original detection of TYLCV in 2011 in the district is also shown in Fig. 1.

For the survey, 100–160 plants were visually inspected per survey site for infected plants. The plants were also randomly sampled and indexed for TYLCV and PLRV using molecular assays. The sampling rates varied depending on the site with 50, 100 or 300 plants sampled (Table 1). Silverleaf whitefly (SLW- *Bemisia tabaci* Middle East-Asia Minor 1) were also sampled by vacuum trapping with 100 suction points within the crop from the survey sites. Numbers of whitefly caught were recorded and whitefly were bulked for TYLCV molecular indexing. The incidence of virus in bulked samples is estimated using a statistical model developed by M. Sharman and based on a formula from Moran et al. (1983) and Rohlf and Sokal (1969).

2.1.2. Alternative host survey

A pre-season survey of major weeds in the areas was conducted in January 2016 to assess the aphid, thrips and whitefly infestation rates. The incidence of the weeds in the environment was recorded, and flowers and major leaves were collected for thrips and whitefly nymph counting.

2.2. Virus indexing

For plant virus indexing, total nucleic acid extracts were prepared using Qiagen Biosprint plant DNA KIT as per manufacturer's instructions, omitting the RNase A from the RLT extraction buffer. Random-hexamer primed cDNA was generated with Superscript III (ThermoFisher Scientific) as per manufacturer's instructions. A duplex PCR was used with 2 mL of the cDNA to test for TYLCV and PLRV, with 5 pmol each of the TYLCV primers TYLCV-F1 and TYLCV-R1 (Brunschot et al., 2010) and 7.5 pmol each of the primers PLRVRdRpF1 (5′-AATCC GGGTT ATGGC TGCCT A-3′) and PLRVCPR1 (5′-GGCCT TGAGT ATTCC ATCCT TGA-3′) (this study). The PCR cycle parameters were as follows: initial denaturation of 95 °C for 30 s, then 35 cycles of 95 °C for 10 s, 62 °C for 20 s, 72 °C for 30 s, and as final extension of 72 °C for 5 min. The TYLCV primers produce a 336 bp band from the C1 gene region and the PLRV primers produce an 881 bp band across the intergenic and coat protein genes. Whitefly DNA was extracted as per Barro and Driver (1997), except that 0.45% (v/v) Triton-X was used instead of 0.45% NP40 in the lysis buffer. Bulked whitefly samples (between five and 20 individuals) were homogenized in 50 µL lysis buffer, and 1 µL used as template in a TYLCV specific PCR as per Brunschot et al., 2010.

2.3. Insecticide trial

A field trial was conducted using a TYLCV-susceptible tomato hybrid (cv. Pinnacle) with different insecticide treatments in a randomised triple replicated small block plot trial. The treatments were: untreated control (T1), 14 mL of Imidacloprid per 100 m row via drip tape application at transplanting (T2), 14 mL of Imidacloprid per 100 m row via plant hole drench application at transplanting (T3), 50 mL of Thiamethoxam/Chlorantraniliprole per 1000 seedlings (seedling tray drench) at transplanting (T4), Foliar sprays of registered products at recommended rates and timing during the season (Actives: pymetrozine, pyriproxyfen, spirotetramat, bifenthrin + piperonyl butoxide) applied during flowering/fruit set (T5), foliar sprays of new currently unregistered chemicals (under development) applied during flowering/fruit set (T6), Best management option program (T7) is a combination of plant hole drench of Imidacloprid (see T3), foliar sprays of registered chemicals (see T5) and new chemicals applied during flowering/fruit set (see T6), TYLCV-resistant hybrid (cv. Red Luck) with currently registered products applied during flowering/fruit set for silverleaf whitefly control (T8). All other insects and diseases were managed using commercial crop protection practices. The SLW population was assessed weekly for the duration of the trial from transplanting (20 August) through to the completion of harvest (17 November). Plants were inspected weekly and the incidence of plants with visual symptoms of TYLCV was recorded. Fruit yield was assessed by harvesting mature fruit from the middle ten plants of each plot every three to four days from 64 days post-transplant (DPT) to 89 DPT. Fruit were counted and weighed and graded as marketable or unmarketable.

2.4. Time of infection with TYLCV

Tomato seedlings were exposed to TYLCV at different times post-transplant to gauge yield differences. Seedlings of the TYLCV-susceptible variety 'Pinnacle' were raised in an insect-proof enclosure at a commercial seedling nursery, then transplanted and immediately covered with insect proof netting. Thirty-six plants per treatment were exposed at 0, 2, 4 and 6 weeks post-transplant to silverleaf whitefly