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Seasonal activity and abundance of *Orosius orientalis* (Hemiptera: Cicadellidae) at agricultural sites in southeastern Australia

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Abstract

Orosius orientalis is a leafhopper vector of several viruses and phytoplasmas affecting a broad range of agricultural crops. Sweep net, yellow pan trap and yellow sticky trap collection techniques were evaluated. Seasonal distribution of O. orientalis was surveyed over two successive growing seasons around the borders of commercially grown tobacco crops. *Orosius orientalis* seasonal activity as assessed using pan and sticky traps was characterised by a trimodal peak and relative abundance as assessed using sweep nets differed between field sites with peak activity occurring in spring and summer months. Yellow pan traps consistently trapped a higher number of *O. orientalis* than yellow sticky traps

Introduction

Orosius orientalis (Matsumura), the common brown leafhopper [synonym = *O. argentatus* (Evans)] belongs to the large and diverse non-endopterygote Hemipteran order and is further classified into the Auchenorrhyncha suborder and Cicadellidae (leafhoppers) family (Day and Fletcher 1994; Dietrich 2005; Fletcher 2009; Oman *et al.* 1990). *Orosius orientalis* is an important vector of many viruses and phytoplasmas in Australia and worldwide. In Australia, *O. orientalis* is responsible for transmitting tobacco yellow dwarf virus (TbYDV, genus *Mastrevirus*, family *Geminiviridae*) to beans (*Phaseolous vulgaris* L.) and tobacco (*Nicotiana tabacum* L.) (Hill 1941; Hill 1950; Thomas and Bowyer 1980; van Rijswijk *et al.* 2002) resulting in economically important production and quality losses of up to 4.5 million AUD annually (Trębicki *et al.* 2007). *Orosius orientalis* also transmits numerous phytoplasmas which cause a range of important diseases including legume little leaf (Hutton and Grylls 1956), tomato big bud (Hill 1941), lucerne witches broom (Helson 1951), potato purple top wilt (Grylls 1979; Harding and Teakle 1985) and Australian lucerne yellows (Pilkington *et al.* 2004a).

Orosius orientalis is active around the borders of tobacco fields where it predominates on several weed species including *Trifolium repens*, *Cryptostemma calendulaceae*, *Plantago lanceolata*, *Malva parviflora*, *Amaranthus retroflexus* and *Raphanus raphanistrum* (Trębicki *et al.* 2009b). Some of these primary host plants act as reservoirs for TbYDV which is acquired by the insect during feeding. When primary host plants become less prevalent the insect moves onto transient hosts such as tobacco and bean, where it transmits TbYDV (Trębicki *et al.* 2009b). Outbreaks of the disease are sporadic and the life-cycle of the predominant vector, *O. orientalis*, on alternate weed hosts, and subsequent acquisition and transmission periods for TbYDV, is poorly understood. *Orosius orientalis* is mainly controlled through the use of insecticides that kill the insect but do not directly control the disease (Paddick and French 1972). However, the timing and frequency of applications has not been optimised. A better understanding of the tritrophic association between the vector, the virus and its host plant should provide an opportunity to improve management practices including reduced and optimised insecticide use, introduction of less toxic insecticide alternatives, or perhaps the use of novel protective agents to reduce the incidence of the disease (Trębicki *et al.* 2009a).

There is currently a paucity of information available on the detection and population dynamics of *O. orientalis* (Helson 1942; Helson 1951; Osmelak 1987). Therefore, as an important first step towards optimising the management of this leafhopper, we gain insights into the population dynamics and seasonal activity of *O. orientalis* under field conditions and evaluated three different trapping methods over two cropping seasons.

Materials and methods

Study sites

The seasonal activity of *O. orientalis* was assessed over two growing seasons, 2005-06 and 2006-07, at four field sites (referred to as A-D) on commercial tobacco farms near Myrtleford in the Ovens Valley region of north-east Victoria, Australia. All four sites have been used for tobacco farming for at least two decades (G. Baxter pers. observ.). Sites A and B were neighbouring farms located in the lower Ovens Valley near Whorouly (36°29'23S, 146°35'48E 210 masl), site C was located between Ovens and Eurobin (36°37'12S, 146°48'31E, 246 masl) while site D was located 6 km from Porepunkah (36°39'46S, 146°14'56E, 320 masl).

Sampling methods

From December 2005 to June 2006 and September 2006 to June 2007, the relative abundance in traps and seasonal activity of *O. orientalis* was monitored at all four sites using three different collection methods, namely yellow sticky trap, yellow pan trap and sweep net. Sticky traps and yellow pans were placed in the field, permanently parallel to the tobacco crop (7-8 metre from the crop border), during two collection seasons and examined at weekly intervals to assess leafhopper activity. At each site, 10 sticky traps and 10 pan traps were used. Every 10 m, one sticky trap and one pan trap was placed along a single 100 m transect. Pan traps were placed between the sticky traps but at a distance of 5 m to the right. Sweep nets were used weekly, to assess leafhopper abundance, in the same plot at a position 5 m to the left of pan traps. Transects at sites B, C and D were aligned NW to SE but due to limited area at site A was aligned NE to SW. Prior to conducting this experiment preliminary studies were conducted collecting traps every three hours during the day and once in the 12 hours during the night over several days to determine leafhopper diurnal periodicity and potential interactions between traps. *Orosius orientalis* was most active in the evening confirming similar observations by Hosking and Danthanarayana (1988) and we did not observe any affects of sweep net on other traps (data not presented). *Orosius orientalis* when disturbed makes only short rapid flights (Helson 1942) which was estimated as 1 m maximum and therefore a 5 m distance was considered a suitable distance to eliminate between-trap interactions. Despite 5 m distance between traps, prior to collecting using a sweep net, water traps were emptied and sticky traps removed and replaced after sweep net sampling.

Sticky traps

Yellow sticky traps (32 cm x 10 cm; Bugs for Bugs, Mundubbera, Australia) were attached to wooden stakes at a height of 20 cm above the plant canopy and were adjusted throughout the season to maintain this height differential. One side of each sticky trap was covered with Tanglefoot®. Using a microspecula and magnifying glass, all leafhoppers were removed from the traps on a weekly basis and transported to the laboratory for counting and identification. Each trap was processed separately and on the same day of collection.

Pan traps

Yellow pan or Moericke traps (Moericke 1951; Moericke 1955) were constructed using round, shallow plastic containers (10 x 34 cm diameter) with a pre-moulded groove on the side to enable draining of the collection fluid. All pan traps ware painted yellow using Dulux® spray paint. All pan traps were placed on the ground and filled to 90% capacity with 5-7% saline fixative solution and a few drops of dishwashing detergent to act as a surfactant. The water level in all traps was checked twice between collection dates and topped up if required. The contents of each trap were processed separately by straining the contents through a fine sieve. Insects were gently washed from the sieve using water and were transferred to a screw-top plastic vial containing 70% ethanol prior to segregation and identification. Leafhoppers were counted using a Grale digital cell counter and identified by morphological characteristics using a stereo microscope.

Sweep nets

Sweep nets (38 cm diameter, fine mesh) were used by swinging the net through the plant canopy at 180° along a 100 m transect where 1 m comprised one sweep. A total of 100 sweeps per site per sampling date were done. The insects collected were placed into a killing jar containing ethyl acetate for 10-15 minutes then transferred into zip-lock plastic bags. To sort samples, four different laboratory test sieves, stacked on top of each other in descending aperture size (3.35 mm, 2 mm, 1 mm and 212 µm), were used to create layers where the sieve with the largest aperture was on the top. The bag contents were emptied to the top sieve and all sieves were shaken for a few seconds. Each sieve was emptied to a white plastic sorting tray and using a magnifying glass and forceps, all leafhoppers were transferred to a Petri dish for identification.

Leafhopper identification

Identification of *O. orientalis* was done using a combination of microscopy and identification keys (Evans 1966; Fletcher 2009; Ghauri 1966). Although *O. orientalis* was mainly identified by its external characteristics and markings, a detailed examination of the internal structure of male genitalia (Oman 1949) was also carried out where necessary to confirm species.

Meteorological data

Meteorological data for both collecting seasons were obtained from the Bureau of Meteorology, Wangaratta, Victoria and SILO database (http://www.bom.gov.au/silo/).

Statistical analysis

Statistical analysis was conducted using Genstat software (10th edn© 2007, Lawes Agricultural Trust). A one-way analysis of variance (ANOVA) was used to compare differences between the numbers of *O. orientalis* trapped using yellow sticky traps and yellow pan traps from September 2006 to February 2007.

Results

Seasonal activity

A total of over 28,000 individual *O. orientalis* were caught from vegetation adjacent to tobacco fields at the four sites (A, B, C and D) using the three different collection methods during two collecting seasons (2005-06 and 2006-07). The total number of *O. orientalis* caught during the 2005-06 tobacco season (December-May) was three-fold lower (n=7046) than that caught in season 2006-07 (September-May; n = 21867) (Table 5.1). Climatic conditions differed between seasons with higher annual rainfall in the 2005-06 season compared to 2006-07 (Table 5.2).

Table 5.1 Number of *Orosius orientalis* recorded from two seasons (05/06 and 06/07) and four sites (A, B, C and D) using three different trapping methods (sweep net, sticky trap and pan trap).

Site	Season					
	2005-2006			2006-2007		
	Trap type			Trap type		
	Sweep net	Sticky trap	Pan trap	Sweep net	Sticky trap	Pan trap
A	81	272	972	114	561	1969
B	403	456	1250	594	1202	3573
C	103	321	2869	1119	1176	8048
D	25	72	222	401	539	2571
Total	612	1121	5313	2228	3478	16161

Table 5.2 Average minimum and maximum temperature and total annual rainfall recorded at the four field sites (A–D) over 3 years

	Year								
	2005			2006			2007		
	Temperature (°C)			Temperature (°C)			Temperature (°C)		
Sites	Maximum	Minimum	Rain (mm)	Maximum	Minimum	Rain (mm)	Maximum	Minimum	Rain (mm)
A & B	21.71	7.63	815.4	23.15	7.1	333.4	22.98	8.88	653.7
C	21.32	7.82	1229.8	22.66	7.44	449.4	22.52	9.07	954.8
D	18.92	6.72	1196.8	20.22	6.47	476.8	20.04	7.86	863.5

Trap evaluation

From all four sampling sites during both seasons, the yellow pan traps caught a four-fold and over seven-fold greater number of *O. orientalis*, respectively, than sticky traps and the sweep net (Table 5.1; Figure 5.1). Furthermore, statistical analyses revealed that there was a significant difference ($P>0.05$) between yellow pan trap and yellow sticky trap data from all sites for each season. Since sweep net sampling was a one-off weekly event, rather than a cumulative weekly sampling event as used with yellow pan and sticky traps, this data was not compared statistically and any comparisons in relative abundance for sampling method should be treated with caution.

Leafhopper population dynamics

Irrespective of trap type, a trimodal peak in *O. orientalis* in activity was generally observed at all four sites (Figure 5.2). However, the relative activity of *O. orientalis* differed between seasons and sites, with a relatively higher activity in the 2006-07 season which was characterised by relatively low annual rainfall and warmer temperatures (Table 5.2). The first population peak occurred early in the season around mid-September (early spring), the second in late November and the third in early January (summer). For the 2006-07 season, the highest activity of *O. orientalis* at sites C and D occurred in the spring, whereas at sites A and B the peak was recorded during the summer. From the end of January till late May, relatively low numbers of *O. orientalis* were trapped at all sites.

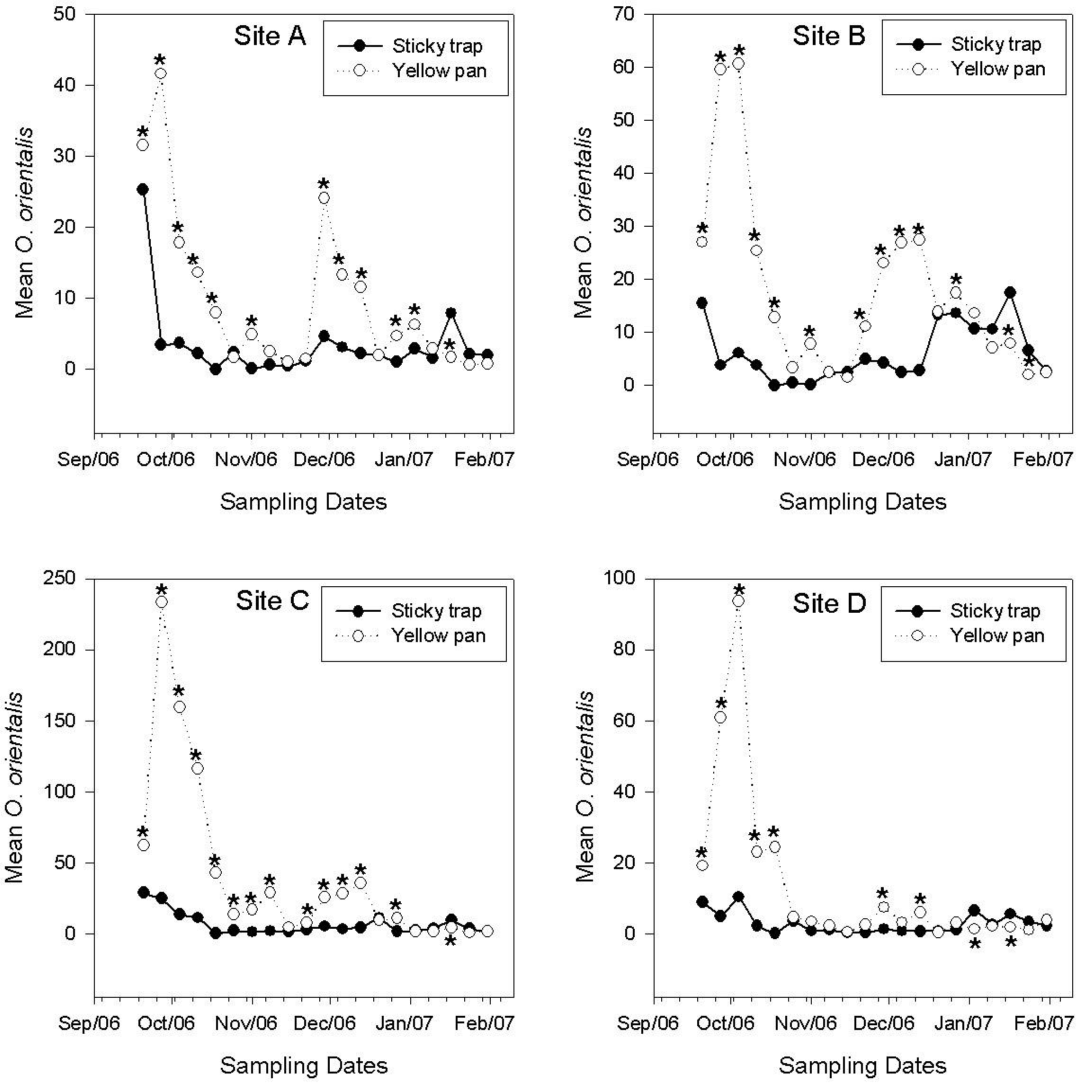

Figure 5.1 Comparison of catches of Orosius orientalis using yellow sticky traps and yellow pan traps from September 2006 to February 2007 from field sites A-D [LSD = 3.64, * P = 0,05].

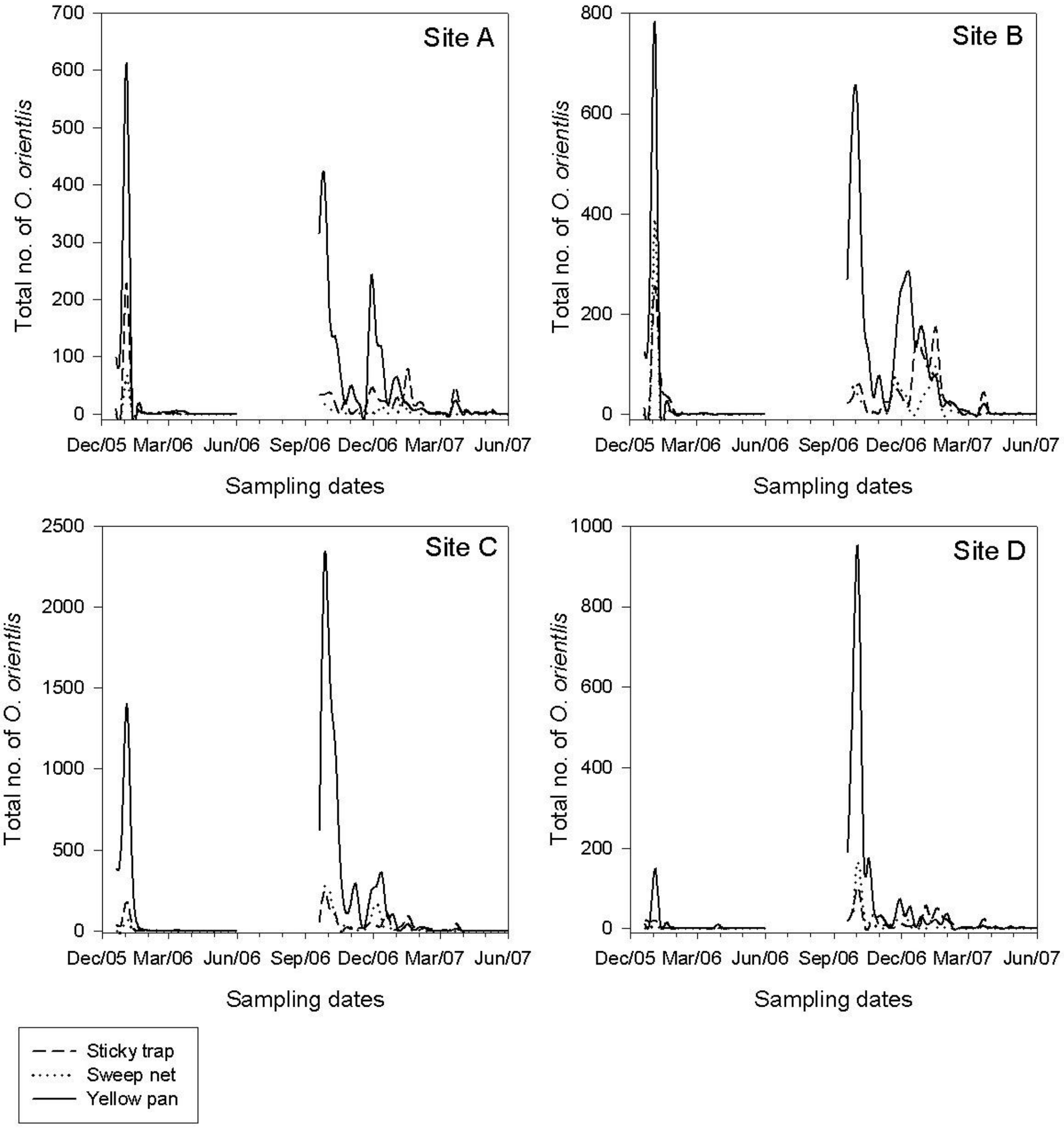

Figure 5.2 Population dynamics of Orosius orientalis over two seasons using three trapping methods at field sites A-D.

Discussion

Although the population dynamics of *Orosius orientalis* have been examined previously under Australian conditions, most of these studies have focused on the association of the leafhopper with lucerne (*Medicago sativa* L.) (Helson 1951; Pilkington *et al.* 2004b) or tomato (*Lycopersicon esculentum* Mill) (Osmelak 1987) and most were carried out over a single cropping season using predominantly light traps and suction traps. In this study, we examined the population dynamics of *O. orientalis* associated with four different tobacco-growing field sites over two seasons using three trapping methods: yellow sticky traps, yellow pan pan traps and sweep nets. *Orosius orientalis* was recorded at all four sites, with peak activity occurring in the spring and summer months. Furthermore, yellow pan traps consistently caught significantly higher numbers of *O. orientalis* than yellow sticky traps.

Previous studies on the population dynamics of *O. orientalis* outside crop areas indicated that the most suitable leafhopper trapping methods were incandescent light traps and suction traps, with water baffle (similar to yellow pan traps), ultra violet light and pitfall traps less suitable (Osmelak 1987; Osmelak and Fletcher 1988). Suction traps have also been used successfully to monitor low level flight activity of *O. orientalis* in grasslands (Hosking and Danthanarayana 1988), whereas studies conducted in sesame fields in Turkey showed yellow sticky traps were more effective than suction traps (Kersting *et al.* 1997). Trap height has also been found to be an important consideration when trapping *O. orientalis* (Pilkington *et al.* 2004a). Although incandescent light, vacuum sampling (Holtkamp and Thompson 1985) and mouth aspirator collection methods were also trialled during the first two months of our monitoring period, these were discontinued in favour of sticky traps, pan traps and sweep nets due to the relatively low catch numbers (data not presented). In contrast to previous studies, we identified three peaks of activity of *O. orientalis* at all four sites. The first population peak occurred in early spring (mid-September), the second in late November and the third in summer (early January). The summer sampling period is particularly important as this is when TbYDV has been shown to be present in both *O. orientalis* and weed species surrounding tobacco fields (Trębicki *et al.* 2009b). In a single tobacco growing season in the Ovens Valley, three generations of *O. orientalis* have been previously observed, first in mid-December, a second in February and a third in mid-March (Helson 1942). Prior to our study, there was limited published data comparing the population dynamics of *O. orientalis* at different sites over successive seasons. Using five different trapping methods over a single season, regular peaks of *O. orientalis* activity have previously been observed in tomato crops from mid-November till February (Osmelak 1987). In a single season study in lucerne, maximum activity of *O. orientalis*, assessed using sweep net catches, were reported in mid-November with high levels till the end of December and smaller peaks in late February and mid-September (Helson 1951). The differences between this study and research conducted previously may be explained by trapping protocol and design as well as climatic conditions. During our collecting seasons, for example, sites experienced conditions of very low rainfall and high temperature.

Between-site differences in *O. orientalis* activity have been reported which are most likely to have been influenced by climate variability and/or vegetation type and diversity (Trębicki *et al.* 2009b). Regardless of these differences, however, the yellow pan trap consistently trapped more *O. orientalis* than either both sticky trap and sweep net. The identification of leafhopper activity peaks is fundamentally important information required to optimise chemical control options for *O. orientalis* and the pathogens it vectors such as TbYDV. It is also important to determine when and by which method, to detect sufficient *O. orientalis* to conduct disease vector studies (Trębicki *et al.* 2009b). In summary, yellow pan traps were found to be the most effective method for trapping *O. orientalis* and characterising population dynamics in this study. However, for practicality, it may be more prudent to use less time consuming approaches in peak periods of *O. orientalis* activity, such as sweep nets which do give a good indication of leafhopper abundance and are relatively simple to use, particularly for vector studies where the requirement is to collect fresh insect samples for virus testing.

The results of this study have provide important information which can be used to develop more effective disease control strategies for *O. orientalis* not only in Australia but also worldwide. *Orosius orientalis* transmits a range of economically important viruses and phytoplasmas in a variety of crops (Harding & Teakle, 1985; Helson, 1951; Hutton & Grylls, 1956; Kersting *et al.*, 1997;

Osmelak, 1986). For example the seasonal activity data from this study when linked to pathogen studies will help determine the most appropriate times for insecticide applications lead to improved control strategies for this economically important vector.

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