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DIVERSITY OF CICADELLIDAE IN AGRICULTURAL PRODUCTION AREAS IN THE OVENS VALLEY, NORTHEAST VICTORIA, AUSTRALIA

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Abstract

There is a paucity of data on the distribution of Cicadellidae (leafhoppers) in Australia. This study quantifies the relative abundance, seasonal activity and diversity of leafhoppers in the Ovens Valley region of Northeast Victoria, Australia. Species diversity and abundance was assessed at four field sites in and around the field borders of commercially grown tobacco crops using three sampling techniques (pan trap, sticky trap and sweep net). Over 51000 leafhopper samples were collected, with 57 species from 11 subfamilies and 19 tribes identified. Greater numbers and diversity of leafhoppers were collected in yellow pan traps. The predominant leafhopper collected was *Orosius orientalis* (Matsumura). Twenty-three leafhopper species were recorded for the first time in Victoria and eight economically important pest species were recorded. Seasonal activity of selected leafhopper species, covering two sampling seasons, is presented.

Introduction

Leafhoppers (Hemiptera: Cicadellidae) are herbivorous insects and many species are economically important pests of agricultural crops in Australia and worldwide (Day & Fletcher 1994). These sap-sucking pest species cause serious damage either directly through feeding or indirectly by transmitting plant pathogens including viruses and phytoplasmas (Backus *et al.* 2005; Gray & Banerjee 1999; Grylls 1979; Weintraub & Beanland 2006).

One of the most important and relatively well-studied leafhopper vectors in Australia is *Orosius orientalis* (Matsumura) which has a broad host range (Trębicki *et al*. 2010). It is a key 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 (*Phaseolus vulgaris* L.) and tobacco (*Nicotiana tabacum* L.) (Hill 1941; 1950; Thomas & Bowyer 1980; van Rijswijk *et al.* 2002), resulting in economically important losses due to diseases (Trębicki *et al.* 2010a). It also transmits numerous phytoplasmas which cause a range of serious diseases in legumes (Hutton & Grylls 1956), tomato (Hill 1941), lucerne (Helson 1951; Pilkington *et al.* 2004a) and potato (Grylls 1979; Harding & Teakle 1985) and is also associated with the spread of diseases in papaya, and grapes (Beanland, 2002; Padovan & Gibb, 2001). Other economically important leafhopper pests previously recorded in Australia include *Austroasca viridigrisea* (Paoli) which causes leaf distortion and stunting in vegetables (Page 1983; Mensah 1996), *Austroagallia torrida* Evans which is a vector of *Rugose leaf curl virus* (Grylls 1954; Grylls *et al.* 1947) and *Batracomorphus angustatus* (Osborn) a vector of the phytoplasmas which cause tomato big bud and potato purple top wilt disease (Grylls 1979).

Although insecticides have been used to control many pest leafhoppers, their use does not necessarily provide effective disease control (Paddick & French 1972; Paddick *et al.* 1971) since this requires an understanding of the insect population dynamics to ensure the optimal timing of insecticide application (Broadbent 1957; Perring *et al.* 1999). In many instances, however, the most suitable sampling method to monitor insect population abundance and migration has not been determined. This paper describes a study of a diverse leafhopper community in and around tobacco production areas in the Ovens Valley region of Northeast Victoria over two cropping seasons. The main aims of the study were to qualitatively analyse leafhopper diversity, identify the most prevalent leafhopper species and compare the efficiency of three sampling methods to monitor the seasonal activity of both native and pest leafhopper species.

Materials and methods

Study sites

This field-based study was conducted during two tobacco (*Nicotiana tabacum* L.) growing seasons, 2005-06 and 2006-07, at four sites (A, B, C and D) on commercial farms located near Myrtleford in Northeast Victoria, Australia. Tobacco has been the predominant cash crop grown at all study sites for the most part of the twentieth century. Sites A and B were neighbouring farms located in the lower Ovens Valley near Whorouly (36°29'23"S, 146°35'48"E 210 masl), while Site C was located between Ovens and Eurobin (36°37'12"S, 146°48'31"E, 246 masl). Site D was located 6 km from Porepunkah (36°39'46"S, 146°14'56"E 320 masl).

Sampling methods

From December 2005 to June 2006 (season 1) and September 2006 to June 2007 (season 2), the relative seasonal abundance and activity of leafhoppers was monitored at all four sites using three different sampling methods; yellow sticky trap, yellow pan trap and sweep net. Sticky traps and pan traps were used to monitor weekly leafhopper activity and sweep nets were used to monitor leafhopper abundance. One sticky trap coated with Tanglefoot™ and one pan trap was placed every 10 m along a 100 m transect. Pan traps were placed between sticky traps 5 m apart. Sticky traps (Bugs for Bugs, Australia; 32 x 10 cm) were attached to wooden stakes 20 cm above the plant canopy. Ten sticky and ten pan traps were used per site. At weekly intervals, all leafhoppers were removed and transported to the laboratory for counting and identification. Each trap was processed separately and on the same day of collection.

Pan traps (Moericke 1951; 1955) were made from round, shallow plastic containers (10 x 34 cm diameter) with a pre-moulded groove on the side to drain the liquid. All pan traps were painted using yellow Dulux® spray paint. Yellow pan traps were placed on the ground and filled to 90% capacity with 5-7% saline fixative solution and a few drops of commercial dishwashing detergent to act as a surfactant. The liquid level in all traps was checked between sample collections and topped up as required. Traps were emptied weekly, by straining all contents through a very fine sieve, and transferred into 70% ethanol prior to segregation and identification. Each trap was processed separately and the containers with specimens were transferred to a Petri dish with 70% ethanol and counted. Due to the large numbers of leafhoppers from each trap, all counting was done using a digital cell Grale™ counter.

Weekly sweep net sampling was conducted. The samples were collected in the same plot area adjacent to tobacco field using the sampling procedure described by Trębicki *et al*. (2010a; 2010b). Sweep nets (38 cm diameter, very fine mesh) were used by swinging the net through the plant canopy, represented by a range of hosts (see Trębicki *et al*. 2010a); at 180° along a 100 m distance and each sweep was approximately one metre. Any potential influence of sweep net catches on sticky trap and pan trap was minimised as described in Trębicki *et al.* (2010b). One hundred sweeps per site per sampling date were done and trapped insects were placed into a killing jar containing ethyl acetate for 10-15 minutes then transferred into zip-lock plastic bags. To size differentiate samples, five different laboratory test sieves were used. Each sieve was emptied into a white plastic tray and, using a magnifying glass and forceps, all leafhoppers were transferred to a Petri dish for identification and counting using a stereo microscope.

Identification

All adult leafhoppers were identified based on morphological characteristics using identification keys (Evans 1966; Fletcher 2009; Ghauri 1966). For most leafhopper species, a detailed examination of the internal structure of male genitalia was also necessary due to difficulties in identification using external characteristics. For this purpose, the abdominal apex was carefully removed with entomological pins, placed in heated 10% potassium hydroxide to macerate the muscle and soft connective tissue was removed making the internal structures clearly visible (Oman 1949). Reference material of selected described species are housed at DPI Rutherglen and undescribed species at the Orange Agricultural Institute, DPI NSW.

Meteorological data

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

Results

Leafhopper diversity and abundance

Leafhopper monitoring was conducted over two collecting seasons; from December 2005 to June 2006 and from September 2006 to June 2007. During this period, a combined total of 51,427 leafhoppers was recorded from the four sites using three sampling methods. Fifty seven described species represented by 11 subfamilies and 19 tribes (Table 4.1) were identified. Deltocephalinae and Typhlocybinae were the most abundant subfamilies with 31 and 10 species recorded, respectively. In contrast, the Agalliinae, Iassinae, Euacanthellinae and Xestocephalinae subfamilies were each represented by only a single species (Table 4.1).

The most abundant species collected was *Orosius orientalis* (Matsumura) which represented 52% (n=26,954) of the total sample size. Other species collected in relatively high numbers were *Anzygina zealandica* (Myers) (number of leafhopper specimens n=6,505), *Maiestas knighti* (Webb & Viraktamath) (n=6,358), *Xestocephalus tasmaniensis* (Evans) (n=4,482), *Nesoclutha phryne*

(Kirkaldy) (n=3,384), *Arawa pulchra* (Knight) (n=791), *Batracomorphus angustatus* (Osborn) (n=763), *Austroasca viridigrisea* (Paoli) (n=366) and *Balclutha frontalis* (Ferrari) (n=165) (Figure 4.1). In addition, two economically important pest species, *Austroagallia torrida* (Evans) and *Ribautiana ulmi* (Linnaeus) were recorded, albeit in relatively low abundance. The remaining non-pest species were recorded in relatively low numbers (< 60) with 20 species recorded on only one or two occasions and generally only from single sites (Table 4.1). Twenty-three species collected had not been recorded previously in Victoria (Table 4.1), while 15 other, as yet undescribed, species (M. Fletcher pers. comm.) were also collected.

Pan traps recorded the largest abundance of Cicadellidae at all field sites. Over both seasons, 78% of Cicadellidae were collected using pan traps with 13% and 9% recorded using sticky traps and sweep nets, respectively. The greatest species diversity (n=43) was also recorded using pan traps (Figure 4.2), whereas similar species numbers were collected using sticky traps (n=32) and sweep nets (n=33). Nineteen species were common to all monitoring methods (Figure 4.2) while two species, *Horouta* spp and *Scaphoideus* spp, were only recorded using pan traps.

There was some general variation in leafhopper abundance and diversity between sites with sites B and C recording the highest total number of leafhoppers as well as higher species diversity (data not shown). This site–to–site variation may have been influenced by climatic and vegetation differences. Sites A and B had relatively higher average temperatures and lower rainfall than sites C and D. The diversity and abundance of vegetation was also marginally different between sites (Trębicki *et al*. 2010a).

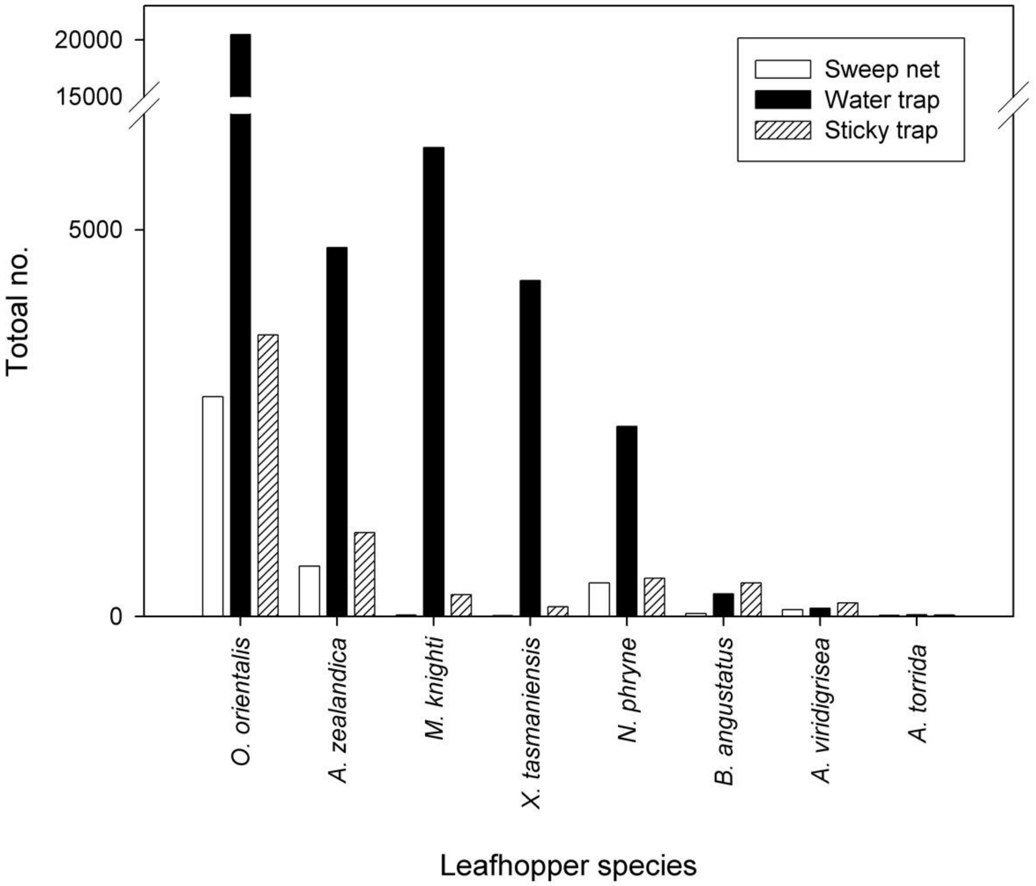

Figure 4.1 Diversity and abundance of leafhopper species collected from four sites, using sweep net, pan and sticky traps, in the Ovens Valley, Northeast Victoria, over two seasons during 2005-2007.

Table 4.1 Diversity and abundance of leafhopper species recorded using three sampling methods (pan trap, sticky trap and sweep net) at four field sites in the Ovens Valley, Northeast Victoria during two collection seasons during 2005-2007.

Subfamily: Tribe	Species	Sampling method		
		Sweep net	Pan trap	Sticky trap
Agalliinae	***Austroagallia torrida* (Evans)**	15	21	19
Deltocephalinae: Athysanini	*Arawa detracta* (Walker)*		16	
	Arawa novella Metcalf*	15	29	
	Arawa pulchra Knight	56	727	8
	Exitianus nanus Distant		2	
	Exitianus plebeius (Kirkaldy)	14	311	20
	Limotettix incertus (Evans)	3	11	10
	Thamnophryne nysias Kirkaldy*	11	16	6
Deltocephalinae: Chiasmini	*Chiasmus varicolor* (Kirkaldy)*	18	31	
Deltocephalinae: Deltocephalini	*Deltocephalus viridellus* Evans*		4	
	Horouta aristarche (Kirkaldy)*		3	

	Horouta austrina (Kirkaldy)*		11	
	Horouta lotis (Kirkaldy)		22	
	Horouta perparvus (Kirkaldy)		594	
	Horouta spinosa Fletcher*		7	
	Maiestas knighti Webb & Viraktamath	16	6062	280
	Maiestas vetus (Knight)	2		
Deltocephalinae: Macrostelini	*Balclutha chloe* (Kirkaldy)*	7	2	1
	Balclutha incisa (Matsumura)	12	9	
	Balclutha lucida (Butler)*	3	1	2
	***Balclutha frontalis* (Ferrari)**	145	9	11
	Balclutha saltuella (Kirschbaum)*	9	6	
	Nesoclutha phryne (Kirkaldy)	434	2457	493
Deltocephalinae: Opsiini	*Opsius stactogalus* Fieber			2
	Orosius canberrensis (Evans)	18	39	
	***Orosius orientalis* (Matsumura)**	2842	20465	3638
Deltocephalinae: Paralimnini	*Diemoides smithtoniensis* Evans	2	4	9
	Euleimonios kirkaldyi Fletcher			5
	Soractellus nigrominutus Evans*		5	
Deltocephalinae: Scaphoideini	*Scaphoideus foshoi* Fletcher & Semeraro*		18	

	Scaphoideus obscurus Fletcher & Semeraro*		3	
	Scaphoideus pristidens Kirkaldy*		6	
Euacanthellinae: Euacanthellini	*Euacanthella palustris* (Evans)*		7	
Eurymelinae: Eurymelini	*Eurymeloides musgravei* (Evans)			16
	Eurymeloides pulchra (Signoret)	7	8	15
Iassinae: Iassini	***Batracomorphus angustatus* (Osborn)**	38	290	435
Tartessinae: Stenocotini	*Smicrocotis obscura* Kirkaldy			2
	Stenocotis depressa (Walker)		2	
Tartessinae: Tartessini	*Alotartessus iambe* (Kirkaldy)*		3	
	Tenuitartessus blundellensis (Evans)*			5
Tartessinae: Thymbrini	*Putoniessa dorsalis* (Walker)	2		17
	Putoniessa nigra (Walker)		8	5
	Putoniessa nigrella Evans	3		22
	Putoniessa rieki Stevens*			4
Typhlocybinae: Dikraneurini	*Aneono australensis* (Kirkaldy)*	7	8	13
	Kahaono pallida Evans	4		16
	Kahaono viridis Evans	7		22
	Kahaono wallacei Evans*			23
Typhlocybinae: Empoascini	***Austroasca viridigrisea* (Paoli)**	86	106	174

	Kybos lindbergi (Linnavuori)*	1	2	
Typhlocybinae: Erythroneurini	*Zygina evansi* (Ross)	9	8	23
	Anzygina sidnica (Kirkaldy)	17	2	
	Anzygina zealandica (Myers)	652	4771	1082
Typhlocybinae: Typhlocybini	***Ribautiana ulmi*** **(Linnaeus)**	2		
Ulopinae: Cephalelini	*Alocephalus ianthe* (Kirkaldy)	2		3
Ulopinae: Ulopini	*Kahavalu gemma* Kirkaldy*		2	
Xestocephalinae	*Xestocephalus tasmaniensis* Evans	12	4343	127

*indicates species not previously recorded in Victoria. Species in bold font represent economically important pest species.

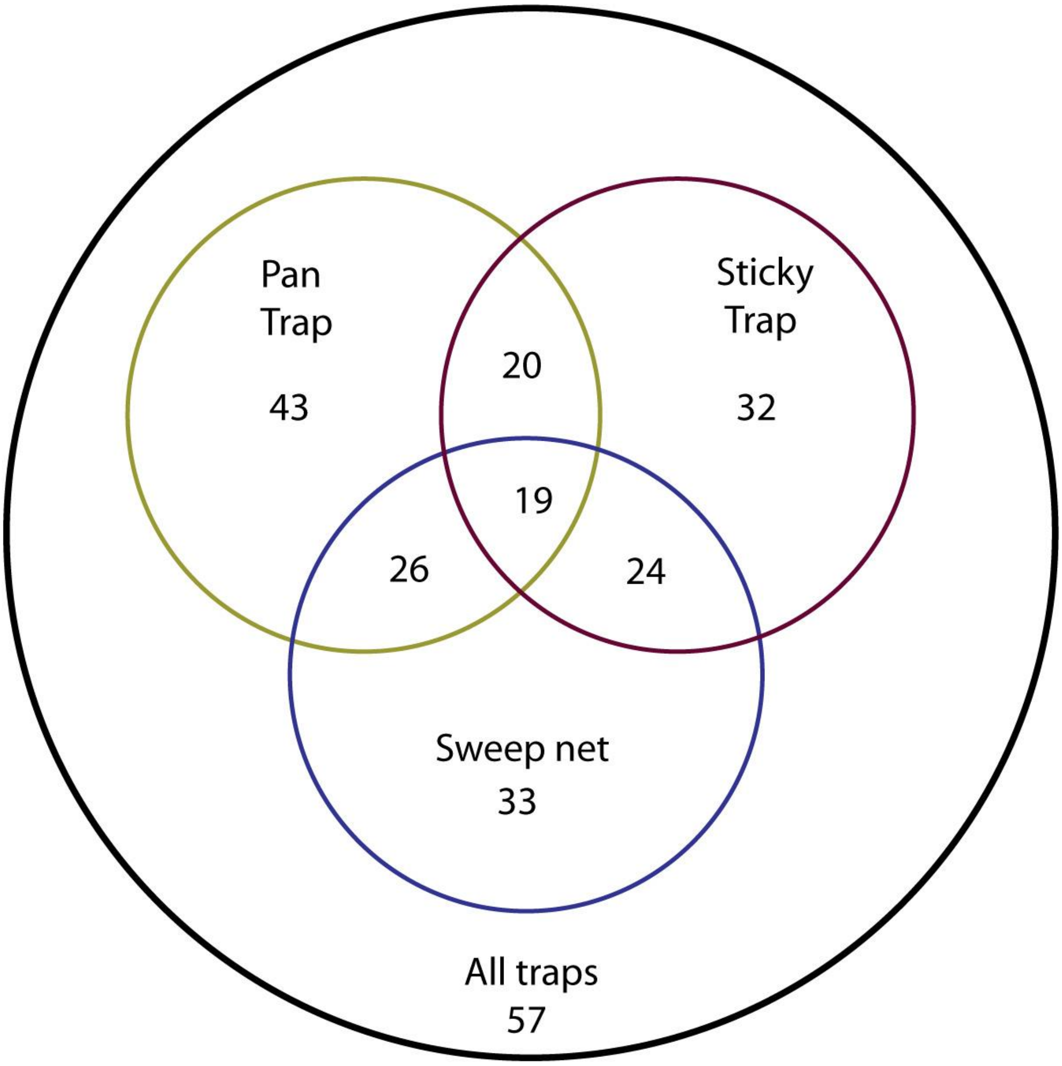

Figure 4.2 Total number of leafhopper species collected over two growing seasons at four sites in the Ovens Valley region, Northeast Victoria, using three sampling methods (pan trap, sticky trap and sweep net). Overlapping areas indicate the number of leafhopper species common for each trap type.

Seasonal leafhopper activity

When insect population data were analysed over two collection seasons, the greatest numbers of native and economically important species (Figure 4.3) were recorded in spring and summer. Generally, trimodal peaks of activity were observed for *A. zealandica, O. orientalis* and *M. knighti*. The initial peak predominantly occurred early in the season during spring (late September), the second late November to December while the third peak occurred in either January to February or March to April depending on the species. For *B. angustatus* and *X. tasmaniensis,* only two peaks of population activity were recorded in November and late January, and December and late April, respectively (Figure 4.3). Apart from *B. angustatus* and *X. tasmaniensis*, most leafhopper species had higher summer peak activity during the first season compared to the second season. Some leafhopper species, however, did not occur in sufficient numbers to show noticeable trends in seasonal activity (data not shown).

Rainfall and temperature data were analysed over the two seasons (2005-2007) to assess the effect of climatic conditions on insect populations. These analyses revealed that average rainfall from all sites was higher during the first collection season (490 mm) compared to the second season (319 mm), while the average maximum temperatures for the first season (24.6°C) were lower than the second season (26.6°C).

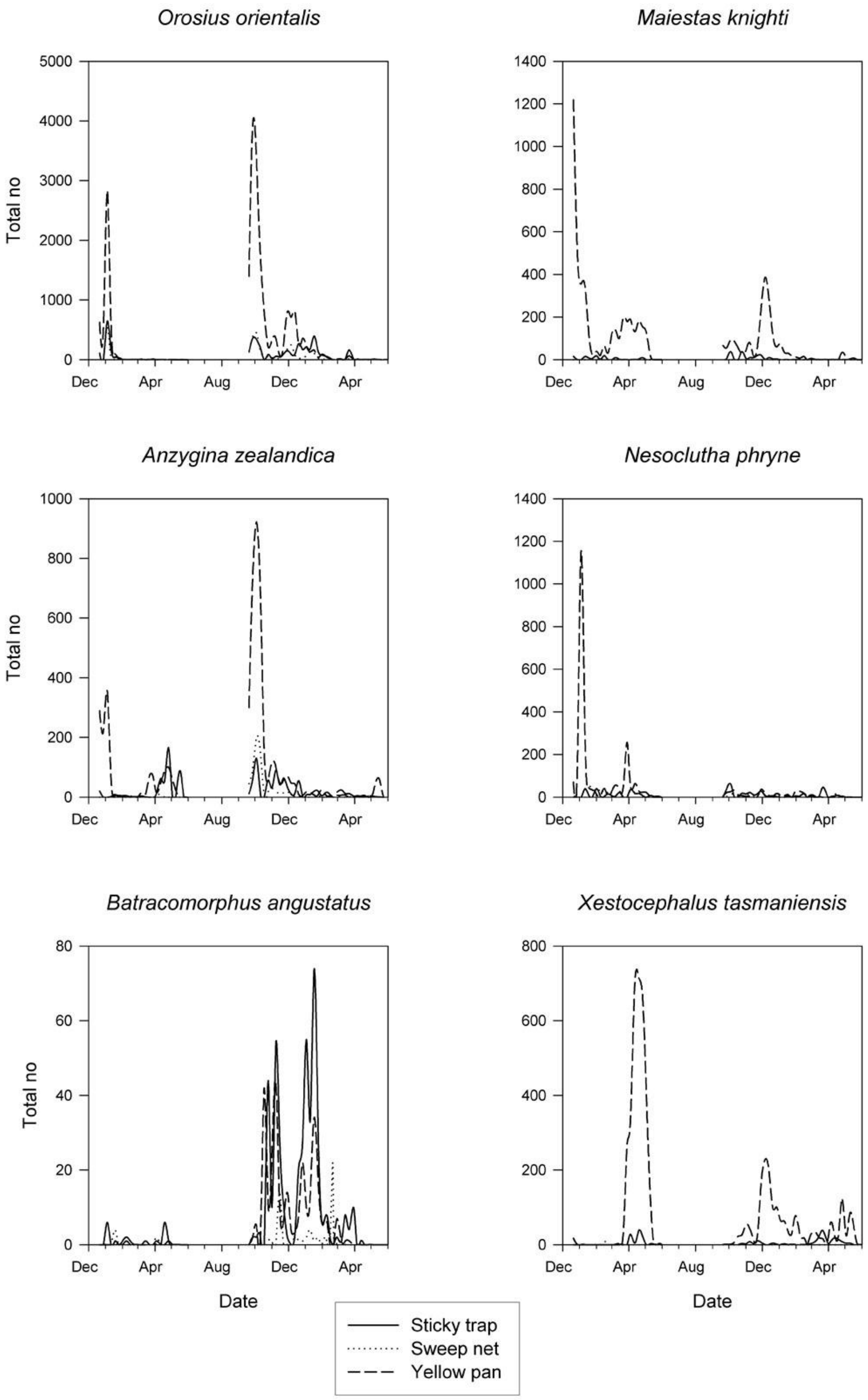

Figure 4.3 Seasonal activity and abundance of six selected leafhopper species collected over two seasons, during 2005-2007 in the Ovens Valley region of Northeast Victoria. Pooled data is shown from four field sites for weekly sample dates.

Discussion

A range of Cicadellidae is known to be present in Victoria, many of which are economically important pest species and vectors of pathogens of economically important crops. This study is the first to quantify the relative abundance and diversity of leafhoppers in the Ovens Valley, Northeast Victoria. The research was conducted on commercial tobacco farms and provided a qualitative and quantitative understanding of the Cicadellidae community in the region. Three sampling methods were utilised over two consecutive growing seasons to maximise insect collection data. *Orosius orientalis* was found to be the most abundant species of leafhoppers collected and is known to be a major economic pest in the region (Trębicki *et al*. 2010b). Several other leafhoppers were also found to occur in relatively high numbers, including *Anzygina zealandica*, *Nesoclutha phryne*, *Maiestas knighti* and *Batracomorphus angustatus*. *Anzygina zealandica* is primarily a grass-feeding leafhopper but has also been recorded on shrubs and trees (Knight 1976; Moir *et al.* 2003) while *N. phryne* (Kirkaldy) = *N. pallida* (Evans) is a vector of *Chloris striate mosaic virus*, *Paspalum striate mosaic virus* and *Cereal chlorotic mottle rhabdovirus* (Greber 1977a; Greber 1977b; Grylls 1963). *Maiestas knighti* and *B. angustatus* are both common in many crops and are important vectors of the phytoplasmas causing tomato big bud and potato purple top wilt (Grylls 1979), respectively. *Austroasca viridigrisea*, a common pest of potatoes, tomatoes, beans, tobacco and lucerne (Page 1979; 1983), was also frequently recorded.

Further taxonomic work is needed for an additional 15 unidentified leafhopper species that were collected in this study, and which are likely to be described species. For example, currently in Australia in the Erythroneurini tribe, there are several undescribed species assigned to *Anzygina* spp., *Empoascanara* spp. and *Pettya* spp. (Fletcher 2009 and updates).

The fact that 41% of all species identified in this study had not previously been recorded in Victoria highlights the limitations of current leafhopper distribution data in Australia. There has been limited research directed towards investigating the population dynamics and diversity of leafhoppers in Australia over the past few decades. Furthermore, this has been restricted to selected crops including lucerne (Helson 1951; Pilkington *et al.* 2004b), tomato (Osmelak 1986; Osmelak & Fletcher 1988) and tobacco (Helson 1942; 1950; Hill 1941; Hill & Helson 1949; Trębicki *et al.* 2010a) with most studies targeting a single leafhopper species, particularly *O. orientalis.* This is the first systematic study conducted over successive seasons on a diverse range of Cicadellidae collected from vegetation around the borders of tobacco fields in Northeast Victoria.

Of the three sampling methods evaluated in this study, both the largest number and greatest diversity of leafhopper species was recorded using the pan trap. Despite obvious differences in the numbers of the most common leafhoppers collected, the highest numbers were recorded around the same time in all traps, especially for *O. orientalis*. In a recent study, Trębicki *et al.* (2010b) reported that pan traps and sticky traps were the most suitable methods for the monitoring of *O. orientalis*, while sweep netting is the preferable monitoring option where fresh samples are needed for pathogen testing. Two additional trapping methods, incandescent light and suction trap, have also been proven effective in collecting leafhoppers outside cropping areas (Osmelak 1987). Although these two methods and collecting using an insect aspirator were also used in the first few weeks of the present study, both were subsequently discontinued due to relatively low catch numbers (data not shown). In previous studies on Hemiptera from understorey or canopy plants, chemical knockdown, vacuum sampling and beating have proven more suitable than sticky trap, hand collecting and branch clipping (Moir *et al*. 2005).

In our study, clear differences were found in both the diversity and abundance of leafhopper species detected using sticky and pan traps. Trap positioning, in relation to surrounding vegetation, is likely to be a critical factor in determining the species collected. Pan traps were placed on the ground, whereas sticky traps were raised above the vegetation. As such, larger numbers of leafhopper species displaying short distance jumps and low level flight activity, such as *N. phryne, O. orientalis* and *A. zealandica* species (Hosking & Danthanarayana 1988), might be expected to be trapped in pan traps. This notion is supported by a study which showed that increases in the above-ground height of yellow sticky traps resulted in a decrease in the numbers of trapped *O. orientalis* and *A. torrida* (Pilkington *et al.* 2004a).

Although the widest diversity of leafhoppers was obtained using pan traps, this method was also relatively time and labour intensive with respect to collecting and processing samples. For example, the high temperature and evaporation rates occurring during the summer months not only necessitated the regular top-up of the trap collection fluid but also resulted in the rapid decomposition of some specimens which hindered their identification. The processing of sticky traps was also relatively time consuming and identification of specimens caught in Tanglefoot™ was somewhat difficult. Despite the sweep net proving the least efficient method for collecting most leafhopper species, especially *O. orientalis,* it was the most convenient in terms of ease of collection and sample processing.

Clearly, the use of several trap types is ideally needed to accurately monitor population abundance, seasonal activity and diversity of a number of different leafhopper species. Due to time and economic constraints, however, this option is not always practical, especially for farmers implementing IPM who often target specific insect pests in monoculture environments. Regardless of the trapping method(s) used, an understanding of the abiotic and biotic factors influencing leafhopper species abundance in a particular region will enable the development of targeted insect management strategies and subsequent strategies for effective disease control.

Overall this study highlighted that a diverse range of leafhoppers, are present in the Ovens Valley region and some of predominant species are economically important vectors of phytoplasmas and viruses and pan traps were particularly useful in monitoring seasonal activity.

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