

Leaf Damaged by Nymph of *Cardiaspina albitextura* and *Cardiaspina retator* (Hemiptera: Psyllidae)

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Cardiaspina (Hemiptera: Psyllidae) is one of the most important genera of the Australian Psyllidae, owing to the spectacular damage to *Eucalyptus* spp. The increase of *C. albitextura* and *C. retator* populations in high numbers caused severe damage on leaves of *Eucalyptus camaldulensis*. Both species have similarities, i.e. they occur on the same host plant and prefer mature leaves of *E. camaldulensis* for oviposition and feeding sites. They might thus be more likely to show competition than the less closely related taxa. The purpose of the study was to examine degree of the resource use by measuring the area of leaves damaged by the nymphs of *C. albitextura* and *C. retator*. The results indicated that the nymph of *C. retator* caused damage twice more damages than that of *C. albitextura*. The leaves area damaged by the females of both species was greater than that by the males.

Key words: *Cardiaspina*, *albitextura*, *retator*, leaf, damage, nymph

INTRODUCTION

Psyllids resemble miniature cicadas and are sometimes called jumping plantlice. Over 100 species occur on both native and introduced landscape plants in the United States. Several psyllid species are pests of crops such as pear, potato, and tomato. Each kind of psyllid feeds on only one plant species or closely related group of plant (<http://www.ipm.ucdavis.edu>).

Psyllids have strong jumping legs with enlarged femora. The nymphs may produce white waxy secretions or honeydew. Most species have to be considered as pests of agriculture because they feed on plant juices. Some transmit viral diseases or produce galls. However, some are suitable as biocontrol agents if their host is a weed. *Heteropsylla spinulosa* for instance is used for the control of the giant sensitive plant *Mimosa invisa* in Papua New Guinea (<http://www.fzi.uni-freiburg.de/cardiaspina>).

Lerps are formed from the honeydew excreted by nymphs of the psyllid. In free-living psyllids the honeydew produced remains in liquid form but in lerp insects it hardens on contact with air to form the lerp. Lerps vary enormously in size, shape and design. They may be simple cones, univalves, bivalves, or intricately woven basket or fan shapes. Each species has its own characteristic shape (http://outernode.pir.sa.gov.au/forestry/publications_index/lerps_insects).

Nymphs pass through five stages or moults before becoming winged adults. At every moult the insect withdraws its stylets from the leaf and selects a new feeding site. The new site is usually within the existing lerp but occasionally the insect moves to a new site and constructs a new lerp. The whole life cycle takes approximately 1-2 months but may be longer, depending on temperature (http://outernode.pir.sa.gov.au/forestry/publications_index/lerps_insects; Nukmal 2004).

The adults are without lerps and hardly exceed 5 mm body length. Most species are of brown or yellowish coloration and are good fliers due to their well developed membranous wings. Because of their jumping hindlegs they are also called 'jumping aphids'. The females possess a saw-like ovipositor which helps insert the base of the eggs into the leaf to avoid desiccation. A female can lay between 45 and 700 eggs during her short life-time. These are laid on stalks plugged into the leaf tissue (<http://www.pir.sa.gov.au>).

Eggs hatch within 10-20 days and the young nymphs or "crawlers" move about the host plant searching for a place to settle. They usually settle within 48 hours of hatching. Once settled they insert their stylets (mouthparts) into the leaf and begin feeding and constructing a lerp, beneath which they will feed and shelter until they become adults. Eggs laid in late summer give rise to nymphs which overwinter in that stage and do not become adults until the following spring. They usually live in colonies of mixed stages. Generation times vary with locality and time of year but most species go through several generations per year. There are five or more generations per year in the Tropics (http://outernode.pir.sa.gov.au/forestry/publications_index/lerps_insects; Nismah 2003).

Cardiaspina Crawford (Hemiptera: Psyllidae) is one of the most important genera of the Australian Psyllidae, owing to the spectacular damage it can cause to *Eucalyptus* spp. Periodically, over large areas of southern Australia have been devastated attacks by *Cardiaspina* spp. on eucalyptus (Collett 2000). All known species of *Cardiaspina* cause death of the leaf tissues around the nymphal feeding sites. They cause a severe defoliation of their respective host. Some species cause the necrosis of a leaf, others only its discoloration. Prolonged psyllid outbreaks result in a considerable loss of increment and die-back of the host (<http://www.fzi.uni-freiburg.de/cardiaspina>).

Considerable attention has been given to the ecology of Australian tree-dwelling psyllids, particularly the genus *Cardiaspina*, which has been intensively studied by several researchers. They recorded that at least five species of *Cardiaspina* have populations that increase to very high numbers from time to time (Nukmal 2004).

Previous studies showed that both *C. albitextura* and *C. retator* prefer mature leaves of *E. camaldulensis* as oviposition and feeding sites (Raymond 1997; Nismah 2005). This closely overlap of site use might result subsequently in competition among the developing nymphs. Based on this supposition, the study on intra- and inter-specific competition among psyllid taxa to determine their influence on the spatial variation and density of psyllid populations is needed.

Given their apparently similar choices of oviposition sites, their probably similar use of the host's tissues, their sessile immatures suffer density-dependent mortality the two species seem very likely to compete with one another. How, then, can they coexist? Before approaching that topic, it is necessary to examine the natural resources for which they may compete: food, space or some other, less obvious factors. The other requirement is to examine degree of the resource use by measuring area of the leaf damaged by nymphs of *C. albitextura* and *C. retator*.

MATERIALS AND METHODS

This study was conducted in field (open parkland), and in the constant temperature room (CT room) of 20 ± 3 °C, Zoology Department, La Trobe University Bundoora Campus, Victoria Australia. Preliminary study on various leaves damaged by nymphal of *C. albitextura* and *C. retator* showed that the visible damage on leaves caused by nymphs of *C. albitextura* could be seen after nymphs reached the third instar, but that caused by nymphs of *C. retator* was apparent at the second instar.

To compare the leave area damaged by nymphs of both psyllid in the field, the samples of damaged leaves were taken from the same tree in the field during two seasons (winter and summer) and were evaluated in the laboratory. The visible damaged areas due to different stages of nymphal (third to fifth instars) were selected from the sampled leaves each season. At least thirty damaged areas from each instar were copied and drawn on to plastic sheets. The visible damaged areas due to nymphs that grew in the CT room were measured repeatedly by drawing the visible damaged on to plastic sheets every week until the nymphs emerged to adults. The damaged areas were measured using Adobe® Photoshop® version 5.1 and UTHSCSA Image Tool© for Windows version 2.1.

Analysis of Variance (ANOVA), LSD test, and t test were used in data analysis of this study.

RESULTS

The result showed that leaves damaged by *C. retator* were twice more than those by *C. albitextura*. The LSD-test on leaves area damaged caused by different nymphal stage of the psyllids in the field revealed that the nymphal of *C. retator* caused more damages than those of *C. albitextura* ($P < 0.05$, Table 1 and Figure 1). The results of ANOVA of effects of species, instar and interaction between species and instar on log area damaged due to *C. albitextura* and *C. retator* revealed that species, instars and their interaction affected significantly log area damaged ($P < 0.005$) (Table 2).

The means of area weekly damaged by females and males nymphs of both *C. albitextura* and *C. retator* reared in the CT room was shown in Table 3 and Figure 2. The t-test revealed that in the first three weeks the males caused more damages on the leaves than the females. However, when the male nymphs of psyllids reached the final nymphal stage in the fifth instar, they caused less damage than the females. At the

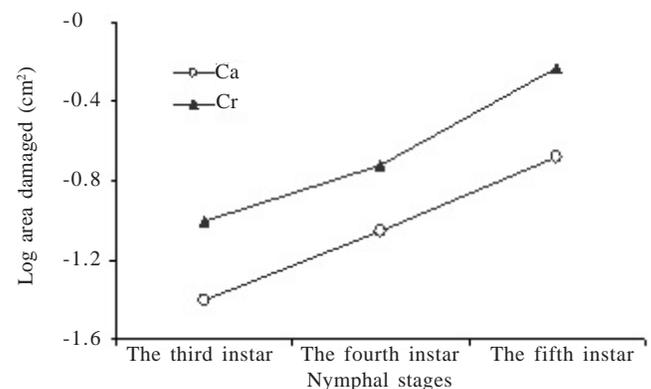


Figure 1. The log area of leaves damaged by different nymphal stages of *C. albitextura* (Ca) and *C. retator* (Cr) in the field.

Table 2. P value of ANOVA on the effects of species, nymphal stages, and interaction of both species and nymphal stages on the log area of leaves damaged by the nymphs of *C. albitextura* and *C. retator* in the field

Source	P
Corrected model	0.000
Intercept	0.000
Species	0.000
Nymphal stages	0.000
Species * Nymphal stages	0.001

* $r^2 = 0.837$ (Adjusted $r^2 = 0.836$).

Table 1. Means area of the leaves damaged by different nymphal stages of *C. albitextura* and *C. retator* in the field

Nymphal stages	Mean of damaged area on leaves (cm ² ± SD)		Ratio of damaged area of leaves <i>C. albitextura</i> : <i>C. retator</i>
	<i>C. albitextura</i>	<i>C. retator</i>	
The third instar	0.040 ± 0.017a	0.102 ± 0.031b	1:2.55
The fourth instar	0.094 ± 0.35a	0.190 ± 0.077b	1:2.02
The fifth instar	0.216 ± 0.087a	0.592 ± 0.254b	1:2.74

Means in the same rows followed by the same letter are not significantly different ($P > 0.05$) LSD-test.

Table 3. Means area of leaves weekly damaged by female and male of the nymphs of *C. albitextura* and *C. retator* in the CT room

Weeks	Female (cm ² ± SD)		Male (cm ² ± SD)	
	<i>C. albitextura</i>	<i>C. retator</i>	<i>C. albitextura</i>	<i>C. retator</i>
1	0.060 ± 0.032aA	0.062 ± 0.026aB	0.065 ± 0.020aA	0.065 ± 0.021bA
2	0.144 ± 0.111aB	0.122 ± 0.065aA	0.158 ± 0.064bA	0.170 ± 0.091bB
3	0.312 ± 0.185aB	0.269 ± 0.134aA	0.351 ± 0.151bB	0.345 ± 0.184bA
4	0.638 ± 0.317bA	1.267 ± 0.335bB	0.490 ± 0.253aA	0.608 ± 0.287aB
5	0.850 ± 0.465	emerged	emerged	emerged

Means in the same rows followed by the same letter were not significantly different ($P > 0.05$) two-sample *t*-test, the small letters are between sex within species and the capital letters are between species within sex.

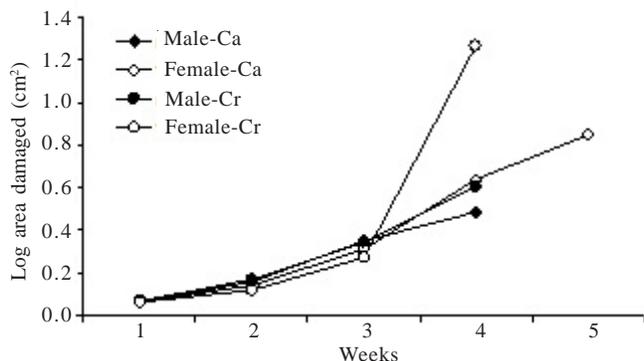


Figure 2. The rate of leaves area weekly damaged by male and female nymphs of *C. albitextura* (Ca) and *C. retator* (Cr) in the CT room.

third week, both the male and female nymphs of *C. albitextura* caused more damage than those *C. retator*. In spite of that, the fifth instars of *C. retator* damaged larger area of leaves than that of *C. albitextura*. The ANOVA shown that species, sex and interaction of both species and sex significantly affected log area of leaf weekly damaged occurred in the CT room ($P < 0.005$) (Table 4).

The third- and fourth-instar of both *C. albitextura* and *C. retator* damaged larger area on the leaves in winter than summer. However, the fifth-instar of those species caused larger area of damages on the leaves in summer than winter (Table 5). The nymphs of *C. retator* caused more damages than those of *C. albitextura* in both winter and summer.

DISCUSSION

The nymphs of *C. retator* produced more damages than those of *C. albitextura*. This suggested that the nymphs of *C. retator* have greater ability to inject a toxic salivary secretion into the phloem than those of *C. albitextura*. Nymphs of *C. albitextura* appear to inject small amounts of a toxic salivary secretion into the phloem, which diffuses into the surrounding tissue. This substance induces a breakdown of the mesophyll

Table 4. P value of ANOVA on the effect of species, sex and interaction of both species and sex on the log area of leaves damaged by male and female of the nymphs of *C. albitextura* and *C. retator* in the CT room

Source	P
Intercept	0.000
Species	0.000
Sex	0.000
Species * Sex	0.000
Error	

tissue, resulting in the liberation of great amounts of soluble nitrogen at the feeding site. The increased availability of soluble nitrogen, particularly amino acids, in the phloem tissue is vital since it determines the rates of growth and survival among the nymphs of psyllids (<http://www.pir.sa.gov.au>). Crawford and Wilkens (1996) found that the most conspicuous change in the leaf tissue affected by nymphs of *C. retator* was the breakdown of the chloroplast internal structure coupled with an accumulation of electron-dense bodies (plastoglobuli). These electron dense bodies consist of a large amount of lipids and proteins and arise as a result of accumulated products of cell membranes and pigments.

The toxin can turn the leaf to red, purple, or brown, and eventually die before the adult insect emerging. Visible damage ranges from localized necrosis of plant tissue to severe damage of leaf and malformation of meristematic tissue. Feeding by nymphs of *Cardiaspina* induced a condition in leaf tissue that resembled advance senescence (Nukmal 2004).

The ability of the nymphs of *C. retator* used the food resources were higher than those of *C. albitextura*. It may relate to the size and growth rate. The nymphs of *C. retator* were slightly bigger than those of *C. albitextura* and their growth rate were twice of those of *C. albitextura*. Faster growth may synchronise with faster damage (Nukmal 2004). Histological examinations of leaves that have supported *Cardiaspina* nymphs show that the chloroplasts in palisade cells had nearly disappeared and the cytoplasm stained very lightly due to coagulation (<http://www.dpi.vic.gov.au>). More

Table 5. Means damaged of area produced by different nymphs stage of *C. albitextura* (Ca) and *C. retator* (Cr) between winter and summer in the field

Instars	Mean damaged of area (cm ² ± SD)			
	Winter-Ca	Summer-Ca	Winter-Cr	Summer-Cr
3	0.074 ± 0.017bA	0.053 ± 0.010aA	0.136 ± 0.030bB	0.081 ± 0.020aB
4	0.126 ± 0.029bA	0.108 ± 0.030aA	0.214 ± 0.080aB	0.196 ± 0.080aB
5	0.227 ± 0.080aA	0.418 ± 0.140bA	0.410 ± 0.150aB	0.544 ± 0.180bB

Means in the same rows followed by the same letter are not significantly different ($P > 0.05$) two-sample *t*-test, the small letters are between season within species and the capital letters are between species within season.

recently, Crawford and Wilkens (1996) found that the most obvious change in leaves fed on by *C. retator* nymphs was the breakdown of the thylakoid membranes in the chloroplasts. At the sub-cellular level, the leaves damaged by psyllids and senescent leaves were resemble one another (Nukmal 2004).

The nymphs of *C. retator* grew faster than those of *C. albitextura*, they probably induced the condition in the leaf tissue which effected their competitor. In phytophagous communities, a species could have a negative effect on others by using plant resources, particularly when the species monopolizes the available plant resources (Nukmal 2004).

The coexistence of *C. retator* and *C. albitextura* decrease population of *C. albitextura*. In contrast, *C. retator* obtained benefits from the presence of *C. albitextura*. They reached greater population size when coexistence with *C. albitextura* than those when the *C. albitextura* were absent. It may indicate that the two species compete with each other such it has been reported by Nukmal (2004). Sap-feeding homopterans can reduce plant nutritional quality and thus have indirect negative effects on co-occurring species (McClure 1980).

The females produced more damage than the males did. It maybe due to the growth rate of females of both psyllid species was higher than that of males. This result supported previous study conducted by Subchan (2005) that growth rate of the female *C. albitextura* in 15-25 °C was slightly higher than that of the male. Sexual dimorphism in adult size could be ascribed to sex differences in the size of insects (Tamaru 1998). Females were bigger than males. Reproductive capacity of insects depend on the females fitness (Sopow & Quiring 1998), and it is generally accepted that fecundity increased with the increase of body size (Honek 1993).

Season, species and nymphal stage significantly affected the damages on the leaves. The areas of leaves damaged by the third and fourth instars of the two psyllids were larger in winter than summer. However, the fifth instar caused less damage on the leaves in winter than summer. This apparently indicated that the need to feed was different in the times and nymphal stages. The nymphs responded to the lower temperature in the winter by prolonging the period of growth. For example, the growth period of the nymphs of *C. retator* was 107 days in winter, and 31 days in summer (Nukmal 2004).

The insect development tend to decrease in low temperature. It was recorded that the development of *C. albitextura* from the egg to the winged adult took 140 days in about 7.1 °C daily temperature of natural condition. However, the period to develop in 20-35 °C daily temperature was only 22 days. There is no true hibernation. In spite of that, the first instar nymphs of *C. densitexta* which hatch and settle early in the winter grow scarcely at all until the following spring (<http://www.pir.sa.gov.au>).

The fifth-instar caused larger damage on the leaves in the summer than winter. It could be assumed that the summer was more favourable for fifth-instar to grow. The nymphs of *C. albitextura* reared in 20 ± 3 °C of CT room grew about three times faster than those grew in 12.1 ± 2.4 °C during winter-spring. Whereas the nymphs of *C. retator* grew five times

faster in 19.1 ± 3 °C of spring-summer than those grew in 11.0 ± 2.5 °C winter-spring (Nukmal 2004). It was suggested that temperature affected the growth rate of both psyllids. These results also supported previous studies on *C. albitextura* at different constant temperatures. The growth rate of lerps of *C. albitextura* increased over a ranged on constant temperature between 15 and 25 °C. The nymphs grew about six times faster at 25 °C than those at 15 °C, but the growth rate decreased at 29 °C. The optimum temperature for the growth of the nymphs of *C. albitextura* was 25 °C (Subchan 2005). The growth rate of nymphs *C. albitextura* was faster within a favourable range of temperatures (20-25 °C) compared to those reared at low temperatures (15 °C) (Subchan 2005). Faster growth may synchronise with faster damage and vice versa, because feeding by nymphs of psyllids induces a condition in the leaf tissue that resembles advance senescence (Denno *et al.* 2000).

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