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Abstract

The lerp-forming psyllid, Creiis lituratus Froggatt, is the most damaging pest of Eucalyptus dunnii Maiden plantations growing in north-eastern New South Wales. During the past 10 years there have been numerous reports that stands of E. dunnii planted on low-lying areas that were prone to waterlogging were also prone to infestation by C. lituratus. The objective of this shadehouse study was to determine whether C. lituratus prefers young E. dunnii exposed to intermittent waterlogging compared with other treatments (drought, normal watering and a control using normal watering plus an insecticide). Also we assessed whether the nutritional status of E. dunnii foliage, in particular amino acid content, differed between watering treatments. Field-collected C. lituratus adults were released into the shadehouse three months after establishing the watering treatments to individually-potted E. dunnii arranged in a 14 × 6 randomised design. Three months after release of the psyllids, we counted significantly more eggs and nymphs present on the plants subjected to intermittent waterlogging compared with the other treatments. Applying general linear modelling (GLM) and Akaike's information criterion (AIC) we found that the best model included both watering treatment and plant structure (through height and diameter), with psyllid infestation (eggs + nymphs) significantly higher on the waterlogged plants and significantly lower in the drought treatment compared with the normal watering treatment. The application of the generalised estimating equations technique to foliar content of individual amino acids and nutrients did not reveal a clear association with watering treatment or psyllid infestation. Most of the significant differences in amino acid content between treatments were between plants watered normally but with or without the systemic insecticide imidacloprid applied as a soil drench. No eggs or nymphal stages were recorded on the plants treated with imidacloprid. This trial demonstrated that the psyllid C. lituratus has a preference for young E. dunnii subjected to periodic waterlogging. Plantation growers can improve their site-species match for E. dunnii by avoiding sites prone to waterlogging.

Keywords

level, infestation, increases, lituratus, psyllid, pest, waterlogging, creiis, dunnii, eucalyptus

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

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Waterlogging increases the infestation level of the pest psyllid *Creiis lituratus* on *Eucalyptus dunnii*

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Summary

The lerp-forming psyllid, *Creiis lituratus* Froggatt, is the most damaging pest of *Eucalyptus dunnii* Maiden plantations growing in north-eastern New South Wales. During the past 10 years there have been numerous reports that stands of *E. dunnii* planted on low-lying areas that were prone to waterlogging were also prone to infestation by *C. lituratus*. The objective of this shadehouse study was to determine whether *C. lituratus* prefers young *E. dunnii* exposed to intermittent waterlogging compared with other treatments (drought, normal watering and a control using normal watering plus an insecticide). Also we assessed whether the nutritional status of *E. dunnii* foliage, in particular amino acid content, differed between watering treatments. Field-collected *C. lituratus* adults were released into the shadehouse three months after establishing the watering treatments to individually-potted *E. dunnii* arranged in a 14 × 6 randomised design. Three months after release of the psyllids, we counted significantly more eggs and nymphs present on the plants subjected to intermittent waterlogging compared with the other treatments. Applying general linear modelling (GLM) and Akaike's information criterion (AIC) we found that the best model included both watering treatment and plant structure (through height and diameter), with psyllid infestation (eggs + nymphs) significantly higher on the waterlogged plants and significantly lower in the drought treatment compared with the normal watering treatment. The application of the generalised estimating equations technique to foliar content of individual amino acids and nutrients did not reveal a clear association with watering treatment or psyllid infestation. Most of the significant differences in amino acid content between treatments were between plants watered normally but with or without the systemic insecticide imidacloprid applied as a soil drench. No eggs or nymphal stages were recorded on the plants treated with imidacloprid. This trial demonstrated that the psyllid *C. lituratus* has a preference for young *E. dunnii* subjected to periodic waterlogging. Plantation growers can improve their site–species match for *E. dunnii* by avoiding sites prone to waterlogging.

Keywords: waterlogging; insects; stress; *Eucalyptus dunnii*; psyllid; *Creiis lituratus*

Introduction

Eucalyptus dunnii Maiden (Dunn's white gum) has a limited natural distribution, occurring in two disjunct populations—one in north-eastern New South Wales (NSW) and the other in south-eastern Queensland. These areas receive high annual rainfall (>1000 mm) and stands tend to occur on fertile, deep and well-drained soils (Boland *et al.* 2006). Since 1994, *E. dunnii* has become the most widely-planted hardwood species in this region, with at least 32 000 ha established, and is favoured for pulp production and some solid timber end-uses (Nichols *et al.* 2008). *Eucalyptus dunnii* is often planted in topographically low areas due to its frost tolerance (Jovanovic *et al.* 2000). Some of the areas planted to *E. dunnii* are low-lying sites with black clays having poor drainage characteristics that are prone to periods of waterlogging and drought (J. Grant, Soil Scientist, Southern Cross University, Lismore, *pers. comm*., November 2006). Waterlogging rapidly leads to anaerobic soil conditions that can limit uptake of water and nutrients by plant roots (Bradford and Yang 1981). In some plant species, therefore, waterlogging causes symptoms similar to drought stress, with restricted water uptake resulting in reduced leaf water potential, stomatal conductance and carbon assimilation (Pryor *et al*. 2006).

Numerous papers in the literature discuss theories related to the predicted enhanced performance and outbreak dynamics of herbivorous insects on water-stressed plants (e.g. White 1969, 1984; Larsson 1989; Price 1991; Waring and Cobb 1992; Koricheva *et al*. 1998; Huberty and Denno 2004). In 1969, T.C.R. White formulated the plant-stress hypothesis which asserts that insect herbivore outbreaks can result from changes in host physiology, specifically an increase in free amino acids, during times of prolonged water deficit. He later expanded this concept, claiming that elevated nutrient content was a common response of plants to a wide range of adverse environmental factors (White 1984). Studies examining this hypothesis, however, have produced variable and often inconclusive results (e.g. Koricheva *et al.* 1998). More recently, Huberty and Denno (2004) proposed that, despite enhanced foliar nitrogen during times of plant stress,

concurrent reductions in turgor and water content interfere with a sap-sucker's ability to access or utilise nitrogen. They modified White's hypothesis (1969) and suggested that phloem-feeding insects, such as aphids and psyllids, respond positively to intermittently-stressed plants but exhibit poor performance on continuously-stressed plants. Therefore, it is important to include an examination of foliar amino acids when examining the potential interactions of tree host responses to adverse site conditions (including intermittent waterlogging) with the risk of additional damage related to biotic agents, in particular sap-sucking insects such as psyllids.

Parallel to the expansion of the *E. dunnii* plantation estate in north-eastern NSW has been the emergence of *Creiis lituratus* Froggatt (Hemiptera: Psyllidae) as the most damaging insect pest of the young eucalypt plantations (Carnegie and Angel 2005; Angel 2007). In 2008, about 20% of the 32 000 ha *E. dunnii* estate planted in north-eastern NSW was estimated to be damaged by *C. lituratus* (A. Carnegie, Forest Science Centre, Industry and Investment NSW, *pers. comm*., July 2009). *Creiis lituratus* is a lerp-forming sap-sucking insect with five nymphal instars, and it typically has four to five generations per year (Angel 2007; Angel *et al.* 2008). Feeding by the nymphal instars causes leaf tissue to become distinctly red/purple in colour and then necrotic (Carnegie and Angel 2005; Angel 2007). High lerp densities result in premature leaf abscission. Population outbreaks of *C. lituratus* can result in crown defoliation, tip dieback and sometimes tree death (Carnegie and Angel 2005; Carnegie *et al.* 2005). Since 1998, the Forest Health Surveillance Unit (for Forests NSW, Department of Industry and Investment NSW) has routinely reported observing *C. lituratus* populations establishing within stands of *E. dunnii* planted on low-lying areas prone to periodic waterlogging (Angel 2007; A. Carnegie, Forest Science Centre, Industry and Investment NSW, *pers. comm*., July 2009).

The objectives of this study were to:

- confirm that *C. lituratus* has a preference for young *E. dunnii* exposed to intermittent waterlogging, but under shadehouse conditions
- determine if the nutritional status of foliage of potted *E. dunnii* differs between watering treatments (intermittently waterlogged, drought and watered normally).

Forest certification schemes advocate moving away from reliance on insecticides (Carnegie *et al.* 2005). Results from this study could be drawn upon by plantation managers to improve their site selection for *E. dunnii* and to apply insecticide only to stands known to be at risk from *C. lituratus* (Carnegie *et al.* 2005).

Methods

Shadehouse trial and treatments

Eighty-four *E. dunnii* seedlings, derived from mixed seedlots and purchased from a local commercial nursery, were transplanted individually into 8 L, 250-mm diameter plastic pots filled with general-purpose potting mix (Hortico®). The pots were arranged into a 14 × 6 grid on a wire table centred in a shadehouse on the Lismore campus of Southern Cross University. The pots were sufficiently spaced so that, after 6 months growth, foliar contact between plant crowns was minimal. The plants were fertilised with Aquasol (Hortico®) and watered routinely before the watering treatments described below were applied. When the plants were about 80 cm in height, a drip irrigation system was set up on the table. Each irrigation dripper provided about 125 ml of water in 5 min, twice daily. Four treatments were applied to the *E. dunnii* plants following a completely randomised design, with the four treatments allocated randomly to 20 pots. The four treatments were:

1. Control (C) = each pot with two irrigation drippers and a single soil inoculation with the systemic insecticide Confidor 200 SC (200 g L^{-1} imidacloprid, Bayer Environmental Science) at a rate of 6 ml diluted to 1 L of water per pot. These plants were therefore neither water-stressed nor infested with psyllids. Imidacloprid is known to bind to soil organic matter, resulting in minimal leaching (Bayer Environmental Science, unpublished results).
2. Normal (N) = each pot with two irrigation drippers. These plants were therefore not water stressed but were subjected to psyllid infestation.
3. Drought (D) = each pot with one irrigation dripper. The drought treatment involved cycles of removing the drippers for three days and then replacing them for three days.
4. Waterlogging (W) = each pot with one irrigation dripper and the pot placed in a plastic container. The waterlogging cycle involved leaving the water in each container for 21 days and then removing the container to allow free drainage for 14 days (total cycle 35 days).

Soil redox potential (Eh) is a simple, indirect estimate of oxygen status. Negative Eh values are associated with anoxic conditions (Rowell 1981). To assess the effectiveness of the waterlogging treatment in establishing anaerobic soil conditions, Eh and pH values were obtained daily for the first 15 days of applying the first cycle of the waterlogging treatment. These measurements were obtained by using a voltmeter (Hanna Instruments, Woonsocket, Rhode Island) and portable Ecoscan pH6 meter (Eutech Instruments Pty Ltd, Singapore). The probes of both instruments were carefully placed into the water of four randomly selected containers holding the potted *E. dunnii*. Three months after establishing the waterlogging treatment, the dissolved oxygen levels were determined using the Winkler titration method (Mitchell and Stapp 1995).

Photosynthetic capacity was assessed by measuring F_v/F_m, the ratio of variable fluorescence to maximum chlorophyll fluorescence, using a portable chlorophyll fluorometer (Fluorescence Induction Monitor 1500, ADC BioScientific Ltd, UK). One clip per leaf was applied for 15-minute dark adaption to five mature leaves on four randomly-selected plants per treatment (four treatments), resulting in a total of 80 leaves being measured. The clips were placed on undamaged fully-expanded leaves to detect only the presence of systemic photochemical stress in the foliage, not direct damage associated with psyllid feeding (Stone *et al.* 2005). These measurements were repeated weekly after the commencement of the watering treatments, including the final watering cycle. The heights and diameters of the *E. dunnii* plants were measured a few days after transplanting, and six months later at the conclusion of the trial. At the end of the trial three plants per treatment were also randomly selected for comparing biomass between treatments.

In February 2008, three months after initiating the watering treatments, *C. lituratus* adults were collected (using a sweep net) from several *E. dunnii* plantations in the Casino region. The adults were transferred to collection jars using a simple aspirator (or pooter), then soon afterwards the opened jars were placed among the potted plants in the shadehouse. Several collections of adults were sequentially released over several days in February 2008. No psyllids were observed on any of the potted *E. dunnii* before the first release. After three months exposure to the psyllids, at the conclusion of the trial, a hand-held magnifying glass was used to visually estimate numbers of eggs, nymphs (individual instars not distinguished), adults and empty lerps. Numbers of insects were scored in multiples of ten. The location of psyllid clusters on each plant was also recorded. Severity of foliar damage was visually estimated at the conclusion of the trial by counting the numbers of leaves per plant within the following broad categories based on the estimated proportion of leaf tissue either discoloured or necrotic: 0–25%, 25–50%; 50–75%, or 75–100%.

Foliar analysis

After three months of treatment application, 20 fully-expanded leaves per plant from three randomly-selected pots per treatment were sampled and immediately frozen using liquid nitrogen for subsequent foliar amino acid analysis. The selected leaves were ovate in shape and undamaged. The samples were transported to the plant physiological laboratory, University of Queensland, St Lucia. In the laboratory samples were broken up and placed into 7-ml PE flat-bottomed tubes (Micronic B.V., Lelystad, Netherlands) along with two small stainless steel ball-bearings, and then shaken for one minute at 27 cycles per second on a mixer mill. After mixing, 0.20 g of the plant tissue were placed into 1.5-mL flip-cap micro-tubes along with 5 µL of 2.5 nM norleucine (an amino acid not found in plant tissues) as an internal standard (Mayland *et al*. 2000). Samples were then suspended in 1 mL of 20% methanol, shaken on a vortex and stored at 4°C overnight. The following morning, the tissue in each tube was condensed into a pellet when centrifuged at 10 000 rpm for 10 minutes at 4°C. A 40 µL subsample of the supernatant was extracted for the amino acid analysis by liquid chromatography using an ultra performance liquid chromatography (UPLC) instrument (Waters Corporation, Rydalmere, Australia) equipped with a BEH C18 1.7 µm 2.1 × 50 nm column and the Acqutag™ derivitisation kit (Waters Corporation). A solvent gradient from a water-based solution across to a 55% acetonitrile solvent was used to separate amino acids in a ten-minute run. Samples were mixed with 120 µL borate buffer and 40 µL of Acqutag derivitising reagent before being analysed by UPLC. Results were recorded as micrograms per gram of dry tissue.

From a further set of four randomly selected plants per treatment, 5–6 fully expanded, undamaged leaves were sampled per plant for foliar nutrient analysis. The leaves were analysed by the Southern Cross University Environmental Analysis Laboratory following accredited agricultural foliar testing methodologies (Ken O'Brien, Operations/Quality Assurance Manager, Environmental Analysis Laboratory, Southern Cross University, *pers. comm*., April 2008). Total nutrient concentrations (nitrogen, phosphorus, potassium, sulphur and carbon) were determined using standardised acid digest/combustion techniques. Total salts (calcium, magnesium and sodium) were determined using standardised digest methods, as were total metals (copper, zinc, iron, manganese, boron, cobalt, silicon and molybdenum).

Statistical analysis

Data were initially examined to determine if there were any spatial patterns of infestation within the shadehouse relating to the experimental setup by applying Moran's I autocorrelation index (Gittleman and Kot 1990). Euclidean distances were calculated based on the row and column location of each pot. Comparisons of single-plant parameters (e.g. tree heights, diameters, biomass; F_v/F_m and psyllid egg and nymph numbers, and foliar damage) among all four treatments were done using the SPSS for Windows (v. 14.0) and the general linear model univariate option. The analyses described below were conducted in R-package v. 2.6.2 (R Development Core Team 2007) using the 'APE' library (Paradis *et al.* 2004).

In order to examine psyllid response to the four treatments, the estimates of egg and nymph numbers were summed and termed 'infestation'. Adults were not included in this estimate due to their mobility; they often took flight when the foliage was being inspected for the immature stages. To determine whether psyllid infestation was a response to a watering treatment and/or plant structure, in particular tree height or stem diameter, we applied generalised linear models (GLMs) with a Poisson distribution. All possible combinations of these factors were tested, resulting

Table 1. Comparison of the hypotheses using a multimodel inference approach and applying Akaike's information criterion (AIC) to examine the influence of the watering treatments and plant structure (height and diameter) on *Creiis lituratus* infestation rates. Models within two AIC points of the best model are considered to have substantial support while those greater than 10 AIC points from the best model have essentially no support.

Model structure	AIC	ΔAIC
Treatment + Diameter	1793	0.0
Treatment +Height + Diameter	1795	2.0
Treatment + Height + Diameter + Height × Diameter	1795	2.5
Treatment + Height	1885	92.7
Treatment	1896	104
Height + Diameter + Height × Diameter	3229	1436
Height + Diameter	3317	1524
Diameter	3318	1525
Height	3680	1888

in nine hypotheses (Table 1). As no psyllids were found on the plants treated with the control treatment (normal watering + systemic insecticide) this treatment was excluded from the GLM analysis. To compare the relative merits of the nine hypotheses tested (Table 1) a multi-model inference framework (Burnham and Anderson 2002) was applied using Akaike's information criterion (AIC) (Akaike 1973) to identify the best or best set of models. AIC trades off model fit and model complexity, with the best model having the lowest AIC value. Models within two AIC points of the best model are considered to have substantial support, those within four to seven AIC points having some support and those more than ten AIC points from the best model having essentially no support (Burnham and Anderson 2002). Concentrations of foliar amino acids and nutrients were compared using generalised estimating equations (GEEs) (Liang and Zeger 1986). GEEs extend GLMs to account for serial correlation related to repeated measures (leaves were sampled from three plants per treatment, so the individual plant was the repeated measure). The response variable was foliar chemical concentration, which was compared across the four treatments, relative to the normal treatment.

Results

Although the average daily redox potential (Eh) values fluctuated noticeably during the first 15 days of waterlogging (range 87 to –134), at the beginning of the cycle the mean value was 87 and at the conclusion of the waterlogging treatment it was –8, with an average value of –15. The pH values for the waterlogging treatment (W) ranged from 6.75 to 7.81, with an average reading of 7.17. The mean level of dissolved oxygen in the water collected in containers at the conclusion of the final waterlogging treatment was 0.42 ppm (range 0.16–0.48). These results indicate that the water in the containers of plants receiving the waterlogging treatment was depleted of oxygen. The mean chlorophyll fluorescence parameter F_v/F_m of the waterlogged plants was significantly lower than for the other three treatments (mean $F_v/F_m = 0.78$ for W versus 0.808 for D, 0.809 for N and 0.81 for C; $n = 20$, model standard error (SE) = 0.005; $P = 0.05$).

At the conclusion of the trial the *E. dunnii* plants were, on average, 1.66 m high (SE = 0.31, $n = 72$). The drought-treated plants appeared to be the smallest but not significantly so ($P > 0.05$). A similar and non-significant trend in plant diameters was found among treatments. In addition, the total dry biomass and root : shoot ratio per treatment were also not significantly different. Therefore, although there were minor trends in plant size between treatments, it appears that the watering treatments applied intermittently did not adversely affect the growth of the young *E. dunnii* over a three-month period.

Psyllid infestation levels

At the time of release, the psyllid adults appeared to settle quickly and eggs were first observed about a week later. Using Moran's autocorrelation index, no spatial patterns in the distribution of psyllid infestation (eggs + all instar nymphs) within the shadehouse were detected ($P = 0.46$) at the conclusion of the trial. Throughout the study, no eggs or nymphal stages were recorded on the plants treated with the systemic insecticide, imidacloprid. At the conclusion of the trial, eggs were observed only on plants treated with intermittent waterlogging and the normal watering regime; no eggs were found on the droughted plants although they had been observed on these plants in earlier visual inspections during the trial (Fig. 1). Significantly more eggs and nymphs were present on plants subjected to the waterlogging treatments compared with the other three treatments (Fig. 1; $P < 0.001$). Although not assessed, it was observed that the waterlogged plants often had more adult psyllids than any other treatment.

The best GLM models (Table 1) examining psyllid infestation levels included both watering treatment and plant structure (through height and diameter). These models were superior compared those in which just treatment or plant size were considered. The best model found psyllid infestation was significantly greater ($P < 0.001$) on the waterlogged plants and significantly less in the drought treatment ($P < 0.001$) when compared with the normal treatment. Infestation also increased proportionally as the diameter of the plant increased ($P < 0.001$) (Table 2). When included in the model, there was a trend in which

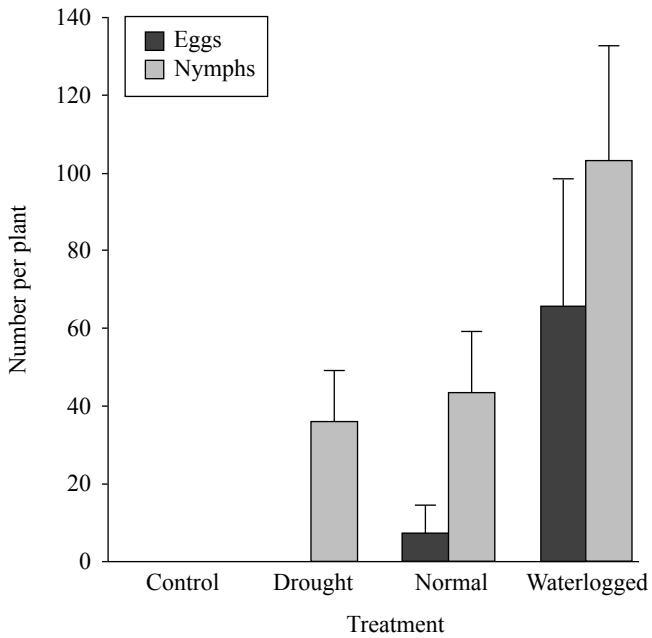

Figure 1. Mean number of *Creiis lituratus* eggs and nymphs found on the young *Eucalyptus dunnii* plants per treatment (18 plants), six months after the commencement of watering and three months after initial release of psyllid adults into the shadehouse. The control treatment is normal watering plus insecticide. The vertical bars represent 95% confidence intervals. The waterlogged-treated plants had significantly more eggs and more nymphs compared to the other three treatments ($P < 0.05$).

Table 2. Using the best GLM model (Treatment + Diameter) to compare psyllid infestation levels on *Eucalyptus dunnii* plants receiving either waterlogged and drought treatments against the normal watering treatment

	Estimate	SE	*z* value	$P(>\|z\|)$
(Intercept)	2.877	0.105	27.4	<0.001
Drought vs normal	–0.412	0.059	–7.0	<0.001
Waterlogged vs normal	1.166	0.043	27.0	<0.001
Basal diameter	0.066	0.006	10.3	<0.001

infestation increased with plant height, but it was not significant ($P = 0.98$).

While the nymphal instars settled on the fully expanded young leaves to feed, about three-quarters of all eggs were laid at the nodes of small branches about half to two-thirds of the way up the plant. Eggs were also found in leaf axils and on the upper surfaces of leaves. Neither eggs nor nymphal stages were found on very young expanding leaves or the oldest mature leaves. Small patches of chlorosis became visible in the presence of second-instar nymphs; these patches turned to a red/purple discolouration by the time the immature nymphs had moulted into the third instar. Necrotic areas of tissue then developed within the purple patches. Psyllid-damaged leaves were present on plants exposed to all three watering treatments (Fig. 2). The waterlogged plants had a greater number of leaves scored as severely damaged by psyllid feeding (> 50% tissue damaged per leaf), but this trend was not significant ($P > 0.05$) when that treatment was compared with the other watering treatments (Fig. 2).

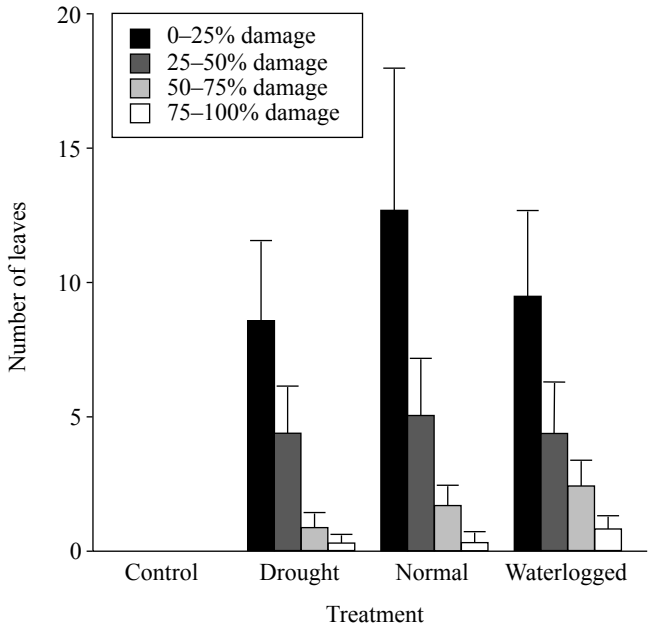

Figure 2. A comparison between watering treatments of foliar damage on the young *Eucalyptus dunnii* assessed three months after initial release of adult psyllids into the shadehouse. The control treatment is normal watering plus insecticide. The figure presents mean numbers of leaves per plant within the following broad categories scoring the proportion of leaf tissue either discoloured or necrotic: 0–25%, 25–50%; 50–75% or 75–100%. Vertical bars represent 95% confidence intervals; number of plants per treatment = 18. The treatment means are not significantly different ($P > 0.05$).

Foliar nutrition

The GEEs revealed a number of significant differences among treatments in the foliar amino acid concentrations but not in the total amino acids relative to the normal treatment (Table 3). Most of these differences, however, were between the insecticide treatment and the normal treatment, where significant differences ($P \leq 0.05$) were recorded for four amino acids (i.e. histidine, serine, aspartic acid, glutamate and tyrosine) and marginal differences ($P \leq 0.10$) for a further three (i.e. alanine, proline and phenylalanine). The imidacloprid-treated plants had significantly less histidine, aspartic acid, glutamate and tyrosine. In contrast,

Table 3. Results of the generalised estimating equations (GEE) analysis comparing foliar amino acid concentrations (micrograms per gram dry tissue) of the normal watering treatment with the three other treatments. Results are presented as means ± standard error (*P* value). Results significant at the $P = 0.05$ level appear in bold type; marginally significant probabilities are denoted with an asterisk.

Amino acid	Insecticide vs normal		Drought vs normal		Waterlogged vs normal	
Histidine	**–8.6 ± 2.4**	**(< 0.01)**	1.7 ± 1.7	(0.33)	**–2.7 ± 1.4**	**(0.05)**
Arginine	22.8 ± 19.2	(0.23)	2.6 ± 3.3	(0.42)	2.8 ± 8.8	(0.75)
Hydroxy.proline	–3.3 ± 10.9	(0.76)	2.0 ± 14.3	(0.89)	–9.3 ± 9.7	(0.33)
Asparagine	–0.5 ± 4.2	(0.9)	3.0 ± 4.7	(0.53)	2.4 ± 2.8	(0.39)
Serine	**–22 ± 4.4**	**(0.01)**	–1.0 ± 4.4	(0.83)	–7.8 ± 9.5	(0.41)
Glutamine	6.1 ± 4.7	(0.19)	–0.1 ± 0.1	(0.31)	–0.1 ± 0.1	(0.22)
Glycine	–0.9 ± 1.4	(0.49)	0.7 ± 1.6	(0.67)	–0.2 ± 1.8	(0.92)
Aspartic.acid	**–26.6 ± 6.6**	**(0.01)**	0.0 ± 9.7	(1.00)	–5.4 ± 8.8	(0.54)
Glutamate	**–42.9 ± 20**	**(0.03)**	10.1 ± 22.8	(0.66)	–14.6 ± 22.2	(0.51)
Threonine	8.6 ± 9.1	(0.35)	–0.1 ± 1.1	(0.94)	–1.0 ± 1.2	(0.40)
Alanine	–7.2 ± 3.8	(0.06)*	1.8 ± 4.7	(0.70)	–6.6 ± 4.0	(0.10)*
Proline	–2.5 ± 1.5	(0.09)*	–2.3 ± 1.4	(0.10)*	1.5 ± 1.9	(0.43)
Tyrosine	**–1.9 ± 0.5**	**(< 0.01)**	0.5 ± 1	(0.67)	–0.4 ± 1.3	(0.74)
Cysteine	–2.0 ± 1.3	(0.13)	4.2 ± 4.9	(0.38)	0.7 ± 1.3	(0.58)
Valine	0.0 ± 2.6	(1.00)	**5.4 ± 2.4**	**(0.02)**	1.7 ± 0.9	(0.08)*
Methionine	–5.9 ± 6.2	(0.34)	3.7 ± 7.5	(0.62)	14 ± 14.9	(0.35)
Lysine	3.7 ± 3.2	(0.25)	–0.4 ± 0.3	(0.17)	1.4 ± 1.2	(0.26)
Isoleucine	–4.9 ± 3.1	(0.11)	0.7 ± 3.1	(0.83)	**5.9 ± 3.1**	**(0.05)**
Leucine	0.9 ± 1.2	(0.45)	–0.5 ± 0.3	(0.16)	–0.2 ± 0.4	(0.63)
Phenylalanine	–4.7 ± 2.5	(0.06)*	**–4.9 ± 2.4**	**(0.04)**	**–5.2 ± 2.4**	**(0.03)**
Tryptophan	0.5 ± 0.4	(0.22)	NA		NA	
Total amino acids	–98.4 ± 58.3	(0.09)*	32.7 ± 48.7	(0.50)	–21.3 ± 65.0	(0.74)

significant differences from the normal treatment were recorded only for three amino acids in the waterlogged treatment (i.e. histidine, isoleucine, phenylalanine) and two in the drought treatment (i.e. valine and phenylalanine). With respect to the waterlogged plants, the sampled leaves had significantly less histidine and phenylalanine but significantly more iosleucine compared with foliage from the normal treatment. In addition, the insecticide-treated plants had significantly less foliar ammonium (GEE analysis: -7.0 ± 3.0, $P = 0.02$), while both the droughted and waterlogged plants showed a trend of more foliar ammonium ($P = 0.15$ and $P = 0.73$ respectively).

The results for foliar salts and metals indicated a few trends but none of the comparisons made were significant ($P > 0.05$ for all comparisons). This may have arisen, in part, due to the small sample size per treatment (4 plants per treatment; 5–6 leaves per plant). The drought-treated plants showed a trend towards slightly elevated concentrations of foliar calcium, magnesium, manganese, boron, molybdenum and silicon compared with the normal treatment. No trends were discernible between the foliar nutrients of the waterlogged and the normal treated plants.

Discussion

This study has clearly demonstrated that C. *lituratus*, when provided with a choice of adjacent plants that were subjected to a range of watering conditions, had a preference for young *E. dunnii* growing under conditions of intermittent waterlogging (21 days of pots sitting in anaerobic water followed by 14 days of free drainage). *Eucalyptus dunnii* has been reported to be less tolerant of waterlogging than *E. camaldulensis* or *E. tereticornis* (e.g. Marcar 1993) and therefore we assumed that the waterlogging treatment would have affected physiological functioning to some degree, possibly through reduced photosynthesis, transpiration and stomatal conductance (e.g. Florentine and Fox 2002). The chlorophyll fluorescence parameter F_v/F_m, an indicator of physiological stress, was significantly lower for the waterlogged plants compared to the other treatments. This implies a decrease in the functioning of the photosynthetic apparatus (Stone *et al*. 2005). Close and Davidson (2003) reported a significant reduction in the maximum photosynthetic rate and photochemical efficiency in waterlogged *E. nitens* saplings compared with control saplings. Although the waterlogged young *E. dunnii* were moderately stressed as a result of the waterlogging treatment applied in our study, the overall effect on growth over the six-month period seems to have been minimal. In fact, the best-performing model for *C. lituratus* infestation rates included plant size (height + diameter) as well as watering treatment (Table 1). We assume that larger plants would provide more sites for oviposition. Our results appear to support the observations of Mopper and Whitham (1992) and Huberty and Denno (2004) who claim that, for phloem feeders such as psyllids, the optimum host is one that is intermittently released from chronic stress.

The severity of psyllid infestation is influenced by several factors including arrival rate of adults, oviposition success of females and nymphal settling, feeding and survival. Our results indicate that *C. lituratus* females preferentially selected waterlogged *E. dunnii* for oviposition and that under this condition there is good survival of the nymphal stages (Fig. 2). Pre-alighting recognition by host-seeking gravid females is based on olfaction and vision cues at both the crown and leaf scales. Finlay-Doney and Walter (2005) proposed that the number of psyllid (*Heteropsylla cubana*) eggs present on *Leucaena* sp. (a legume crop) is a function of psyllid arrival rates on the plants rather than a direct reflection of the suitability of the substrate for nymphal performance.

If *C. lituratus* females can detect and respond to localised gradients in relative humidity this might have influenced the oviposition rates observed on the potted *E. dunnii* (e.g. Sipura *et al*. 2002). Many eucalypt species are known to close leaf stomata as a mechanism to reduce moisture loss through transpiration (e.g. Michelozzi *et al*. 1995). Feeding psyllid nymphs often insert their stylets through stomatal pores to access the vascular tissue (Woodburn and Lewis 1973) and this may account for the reduced numbers observed on the droughted *E. dunnii* plants. Relative humidity and moisture stress can also influence foliar anatomical characteristics, in particular epicuticular wax production and composition (e.g. Koch *et al*. 2006; Shepherd and Griffiths 2006). Steinbauer *et al*. (2004) reported that epicuticular waxes on *E. globulus* foliage acted as an oviposition stimulant for the autumn gum moth (*Mnesampela privata*), while Brennan and Weinbaum (2001) reported that leaf waxiness of *E. globulus* affected the number of stylet tracks reaching the vascular tissue and nymphal survival of the two free-living psyllids, *Ctenarytaina eucalypti* and *C. spatulata*. We suggest that an examination of epicuticular waxes of *E. dunnii* growing under a range of soil moisture conditions might provide insight into possible cues influencing the settling and feeding success of *C. lituratus*. Another plant response associated with waterlogging is the build-up in the roots of 1-aminocyclopropane-1-carboxylic acid (ACC), which is the immediate precursor of the phytohormone ethylene (e.g. Bradford and Yang 1981; Tang and Kozlowski 1984). It has recently been suggested that ethylene can act as a host location cue for some insect herbivores (e.g. Ramos *et al*. 2008). Perhaps *C. lituratus* adults can detect localised gradients in ethylene concentration?

Our shadehouse study provided only equivocal support for White's plant-stress hypothesis (1969). The elevated numbers of *C. lituratus* on waterlogged *E. dunnii* were not associated with elevated foliar nutrients, in particular amino acid content. While there were significant differences in foliar concentration between the watering treatments for a few specific amino acids, no clear association between watering treatment and foliar nutritional status was detected. The accumulation of amino acids such as proline, asparagine and glutamine has been reported for numerous plant species exposed to moisture stress (Stewart and Larher 1980), but this usually occurs in the presence of severe moisture deficit and visible wilting. These authors report an accumulation of amino acids such as gamma-amino butyric acid, alanine, glycine, serine and proline but decreases in glutamate, aspartate and glutamine in the foliar tissue of plants responding to flooding. Our plants did not present symptoms of visible wilting or shoot death so were probably not moisture stressed. The absence of a significant increase in known osomoregulatory amino acids such as proline also suggests that the plants were not severely moisture stressed. In addition, Close and Davidson (2003) reported that long-term oxygen deprivation through waterlogging can significantly reduce foliar calcium, potassium and phosphorous in *E. nitens* saplings

but significantly increases manganese and iron content. We did not confirm these results in our trial with *E. dunnii* but the small sample size for the foliar mineral analysis may have contributed to this discrepancy.

Interestingly, the comparison between plants treated with the systematic insecticide imidacloprid and those treated with a normal watering regime revealed the largest number of significant differences in amino acid content. One of the degradation products of imidacloprid is 6-chloronicotinic acid which is thought to act as a defensive priming chemical involved in the induced systemic resistance metabolic pathway in tomatoes (Thielert 2006; Goellner and Conrath 2008). Bayer CropScience (Germany), who developed imidacloprid, claim that this systemic compound induces priming for improved activation of defence responses, thereby enhancing their tolerance to biotic and abiotic stresses.

In the field, waterlogged eucalypts, including *E. dunnii*, often present red/purple-coloured foliage in the absence of insect herbivory (e.g. Ladiges and Kelso 1977). In our study, however, this discolouration was observed to be strongly associated with nymphal feeding. The presence of salivary enzymes has been identified in several species of aphids (Miles and Peng 1989; Urbanska *et al*. 1998) and some of these salivary enzymes are capable of catalysing the breakdown of cell walls which facilitates access to soluble nutrients (Madhusudhan *et al.* 1994). While we did not observe the death of entire shoots, field observations (e.g. Carnegie and Angel 2005; Angel 2007) do suggest that the phytotoxic saliva of *C. lituratus* might be systemic and can be translocated up into developing shoots. In the presence of high psyllid levels this could result in extensive branch death and even crown mortality.

The results from this shadehouse trial support field observations that *C. lituratus* has a preference for young *E. dunnii* growing in stands that are periodically flooded. We therefore recommend that plantation growers avoid planting *E. dunnii* on sites prone to waterlogging. In our study, the systemic insecticide imidacloprid was very effective at preventing *C. lituratus* infestation of *E. dunnii* when applied as a soil drench. Currently this insecticide is registered in Australia for use in eucalypt plantations as a tablet formulation (Bayer Initiator®) at planting and as an injectable formulation for protecting older trees (Bayer SilvaShield®). The tablets have been shown to be effective against several defoliating insects in *E. globulus* plantations (Collett and McBeath 2007). Another promising systematic insecticide is clothianidin (Sumitomo Shield Systemic Insecticide™). Preliminary trials of this insecticide applied as a soil drench have indicated a residual efficacy of up to two years against *C. lituratus* in young *E. dunnii* plantations (D. Paton, Sumitomo Chemical Australia Pty Ltd, *pers. comm.*, July 2009). Both these insecticides could be used for protecting stands identified at risk from *C. lituratus* attack.

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