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Reducing the immigration of suspected leafhopper vectors and severity of Australian lucerne yellows disease

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Abstract. Three newly-sown lucerne stands in the mid Lachlan Valley region of New South Wales, Australia, were sampled, over 50 weeks, for Australian lucerne yellows disease symptom distribution and severity. Leafhopper populations were also monitored. Symptoms developed in all 3 stands within 32 weeks of sowing. There were statistically significant spatial differences in the density of symptomatic plants for 2 dates at this and another site. Two possible insect vectors, *Austroagallia torrida* and *Batracomorphus angustatus* were more numerous in some sections of crop margins at 2 sites. These 2 species and a third possible insect vector *Orosius argentatus* each had a statistically significant spatial and temporal correlation with symptomatic plant numbers for at least 1 site date. Two subsequent border treatment experiments evaluated the effect of crop-margin treatments on leafhopper movement into and from the stand. The second border treatment experiment examined also the treatment effect on Australian lucerne yellows disease symptomatic plant numbers. Treatment with insecticide or herbicide significantly reduced the overall movement of leafhoppers. In addition, the insecticide treatment lowered the incidence of disease expression in adjacent lucerne. Results suggest that there is scope for management of this plant disease by reducing immigration of leafhopper vectors into lucerne from non-crop vegetation.

Introduction

Lucerne (*Medicago sativa* L.) is considered to be the most important forage crop in the world (Lolicato and Lattimore 1998). It has been used for grazing, conserved fodder and the production of value-added products (e.g. cubes) in Australia since the mid 1920s (Fitzgerald *et al*. 1980). Australian lucerne yellows (ALuY) is a serious disease, impacting heavily on the Australian lucerne seed industry. It causes an estimated annual seed yield loss valued at AU$7 million to the industry (Pilkington *et al*. 1999), although affects on pasture production are not known. The disease has been reported in lucerne since the early 1950s (Anon. 1953), yet little is known of its epidemiology. Symptoms of ALuY include yellow discolouration of foliage and a distinctive yellow to dark brown discolouration immediately under the periderm of the taproot (Pilkington *et al*. 2002).

Recent work has shown that a phytoplasma is associated with ALuY disease (Pilkington *et al*. 2003). This group of plant pathogenic mollicutes is associated with over 300 plant diseases around the world (Davis *et al*. 1988). Phytoplasmas are transmitted exclusively by insects (Hanboonsong *et al*. 2002), specifically leafhoppers (Cicadelloidea), planthoppers (Fulgoroidea) and psyllids (Psylloidea) (Tsai 1979; Ploaie 1981). More than 30 species from these superfamilies have been identified in Australian (Bishop and Holtkamp 1982; Osmelak *et al*. 1989) and American lucerne stands (Sulc *et al*. 2001).

Preliminary surveys of the above insect taxa in the ALuY-affected study area showed the presence only of: *Austroagallia torrida* (Evans); *Batracomorphus angustatus* (Osborn); *Orosius argentatus* (Evans); *Balclutha incisa* (Matsumura) and/or *B. saltuella* (Kirschbaum); *Austroasca viridigrisea* (Paoli) and/or *A. alfalfae* (Evans); and *Zygina zealandica* (Myers) (L. J. Pilkington unpublished data).

As phytoplasmas are restricted to the phloem of infected plants (Guthrie *et al*. 2001), it follows that their vectors feed on phloem (McCoy 1979). *Balclutha* sp., which feeds exclusively on grasses (Knight 1987), and the known parenchyma feeding species, *Austroasca* sp. and *Zygina* sp. (Carver *et al*. 1991), were discounted as possible vectors of ALuY. *Orosius argentatus* has been shown to be responsible for transmission of lucerne witches' broom and has also been implicated in many other phytoplasma diseases, including Australian grapevine yellows (AGY) (Padovan *et al*. 1996). *Austroagallia torrida* is a known vector of viral and bacterial plant diseases (Grylls 1979) and both *A. torrida* and *B. angustatus* have also been suggested as possible vectors of AGY (Osmelak *et al*. 1989). The presence of *O. argentatus*, *A. torrida* and *B. angustatus* in ALuY-symptomatic lucerne stands suggests that these 3 species are possible vectors of this disease.

Information on the spatial and temporal appearance of symptoms is important in understanding the epidemiology of any disease and, when combined with data on densities of

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insect species, is likely to identify potential vectors (Lindblad and Areno 2002). Many plant diseases have a clear association with an insect vector because of their presence in high numbers or a spatial and/or temporal relationship (Zhang *et al*. 2000). For example, alfalfa witches' broom in lucerne is associated with high levels of the 3 leafhopper species *Aceratagallia* sp., *Neokolla hieroglyphica* (Say) and *Cuerna septentrionalis* (Walker) (Khadhair *et al*. 1997). Lindblad and Areno (2002) found that a high over-wintering population of *Psammotettix alienus* (Dahlbom) in non-crop vegetation was associated with subsequent high levels of wheat dwarf virus. Correlations between densities of potential insect vectors and distribution of disease symptoms can, therefore, provide significant clues to help identify principal components of the disease transmission process.

To assist studies of disease–vector relationships, it is also useful to consider information such as the spatial distribution of symptomatic plants (Arnò *et al*. 1993) and to combine this with the spatial distribution of potential vectors (Ioannou and Iordanou 1985; Grilli and Gorla 1998; Lindblad and Areno 2002). In many disease systems where the vector is a leafhopper, disease incidence declines with distance from the source of the vector (Purcell 1974). Proximity to host plants of the vector also increases disease incidence (McClure 1980; Grilli and Gorla 1998). Correlating temporal incidence of insect populations with disease expression is also useful in studies of disease–vector relationships. This can identify potential vectors (Groves *et al*. 2001; Elder *et al*. 2002), as a higher incidence of insect numbers is common before a disease outbreak (Mann *et al*. 1996; Lindblad and Areno 2002). Removing insect vectors from non-crop vegetation adjacent to crops, before they are able to transmit the pathogen, offers a means to reduce disease incidence (Grilli and Gorla 1998). Still, some diseases require almost total eradication of the insect vector to successfully manage the disease (Holt *et al*. 1999). Limiting the movement of vectors into a crop may, however, lower the incidence of disease (Chancellor *et al*. 1996; Lindblad and Areno 2002) and this approach presents an opportunity to develop a management strategy for ALuY. In order to establish potential management strategies, an understanding of the biology of the insects involved is essential (Osmelak 1984).

The aims of this study were: (i) to survey 3 lucerne stands over 12 months, to capture, for the first time, information on the aetiology of ALuY disease; (ii) to correlate the spatial and temporal appearance of disease symptoms with the incidence of the 3 most common leafhopper species, to provide a preliminary indication of the potential vector status of each leafhopper species; and (iii) to utilise pesticide treatments to crop-margin vegetation, to measure the extent of disease suppression that may be achieved by reducing vector immigration.

Materials and methods

Symptom and leafhopper survey

Three newly sown certified lucerne (cv. Aurora) seed stands were selected in the mid Lachlan Valley region of New South Wales, Australia. All were less than 6 months old at the start of monitoring and had a density of 20–40 plants/m^2. These irrigated stands were separated by a minimum of 20 km. The area of each stand ranged from 12 to 1 ha. Vegetation adjacent to each field included exotic weeds, native grasses, trees and crops.

Each stand was divided into either 99 or 104 subregions, using a grid format. Width and length were divided into intervals such that, on any power transformation, the subregions were of equal size (Fig. 1), a technique developed for this study. This power transformation was chosen so that, when dimensions were back-transformed, subregions nearer the boundaries, where it was anticipated greater precision in spatial sampling would be required, were smaller than subregions closer to the centre of the stand, where greater homogeneity was assumed.

On each sampling date for insect distribution (details in following section) evidence of symptom expression was monitored. Plant disease

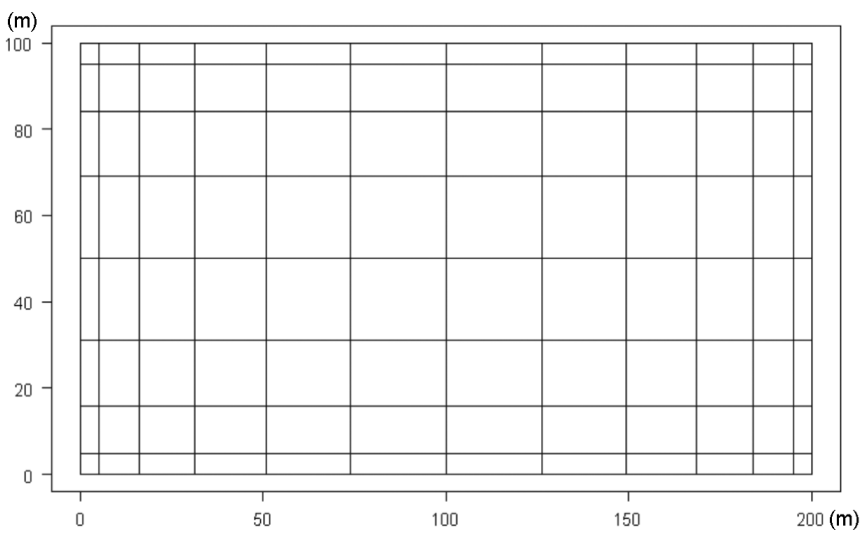

Figure 1. Example of division of a site into subregions.

surveys were initiated at each site at the first appearance of ALuY symptoms. For each of the 3 sites, disease data were recorded from each subregion on the following occasions. Site 1 was sampled monthly, on 3 occasions, after symptoms appeared on 4 January 2001; site 2 was sampled monthly, on 4 occasions, after ALuY symptoms appeared on 22 January 2001; and site 3 was sampled monthly, on 5 occasions, after symptoms appeared on 23 January 2001. The first sample dates for each site were within 32 weeks of the date of sowing for each stand.

On each sampling occasion, a small ball was cast into each subregion, the nearest 100 lucerne plants were identified and the area these plants occupied was measured. The number of plants showing ALuY symptoms was recorded and the severity of the symptoms for each plant was rated on a scale of 1–5 (Table 1). Where there was more than 1 symptomatic plant, within the sample of 100, the distance from each symptomatic individual to its nearest symptomatic neighbour was recorded. Numbers of symptomatic plants, symptom severity scores and numbers of symptomatic plants per square metre were mapped, initially to identify factors with marked spatial trends that merited further analysis. Regression analysis was subsequently made of numbers of ALuY symptomatic plants *v.* the 2 dimensions of the lucerne stand, using the Genstat software package (Genstat Committee 2002).

Insect distribution survey

Leafhoppers were surveyed at all 3 sites, fortnightly in summer and monthly in winter. Sampling was conducted over 12 months from 8 November 2000, including the dates on which ALuY disease symptom data were recorded at each site. Within each subregion, the sample position was determined by casting a small ball into each subregion and then taking a random number of steps (between 10 and 20) in a random direction, so as not to cross the original path of the throw or roll of the ball. This method was adopted to ensure minimal disturbance of the insect population in the immediate sampling area. A circular area of 0.2 m^2 was then delineated by placing a plastic garbage bin from which the bottom had been removed. Insects were collected from this area with a motorised vacuum sampler, as described by Hossain *et al*. (1999). Samples were stored in a portable 12 V car refrigerator at 9°C and returned to the laboratory for identification and counting.

On each sampling date, leafhoppers were also sampled from non-crop vegetation adjacent to each lucerne stand. This was done with the vacuum sampler, but not by bin delineation, because the vegetation included large shrubs. When the plant community was a monoculture, plants at positions about 50 m apart were randomly selected along each crop margin. When the plant community was not a monoculture, a representative of each plant species was sampled along the entire border. At each point, individual plants (if large) or plant community (if consisting of smaller individuals) was sampled for 60 s. Plant species were identified in the field or samples were collected for subsequent identification.

Regression analysis of numbers of leafhoppers caught *v.* row distance and column distance was performed, using Genstat (Genstat Committee 2002), to model the spatial distribution of each species within the stand. A range of regression parameters were used and models that accounted for the most variability were fitted. On 2 occasions (20 November 2000 and 29 December 2000), a 2-dimensional model (insect numbers and distance from a stand edge) was used as this accounted for maximum variance. On all other dates, 3-dimensional models (that included insect numbers and distance from stand edge in 2 dimensions) were used.

Table 1. ALuY symptom severity assessment scale

Symptom level	Symptom description
1	Healthy plant
2	Slight yellowing of foliage No discolouration or drying of stems
3	Severe yellowing of foliage, some reddening Slight yellowing of some stems Drying of leaves or stems
4	Severe reddening Severe drying of foliage or stems
5	Death of plant (root symptom verification)

Border treatment experiment

Given that proximity to vector (Purcell 1974) and vector host plants (McClure 1980) increases the incidence of disease in other systems, the hypothesis that the modification of non-crop vegetation adjacent to lucerne stands would lower incidence of ALuY infection was tested. Insecticide was used to remove insects entirely, particularly leafhopper species. Herbicide was used to remove vegetation.

Experiment 1. Two certified lucerne (cv. Aurora) seed stands were established in the mid Lachlan Valley and separated by 2 km. Both of these irrigated stands had adjacent vegetation consisting of *Echium plantagineum* L. (Paterson's curse), *Trifolium repens* L. (white clover), *Cynodon dactylon* (L.) Pers. (couch grass), *Silybum marianum* (L.) Gaertn (variegated thistle), *Onopordum acanthium* L. (Scotch thistle) and *Chenopodium album* L. (white goosefoot). At each site, a 180 by 10 m strip was marked along a 300 m boundary of the lucerne field. This strip was approximately central on the boundary and the vegetation within it was relatively homogenous for botanical composition and vigour.

The strip on each of the 2 sites was divided into 9, 20 by 10 m plots (3 blocks, each with 3 treatments). The allocation of treatments to plots was undertaken using Spades (Coombes and Gilmour 1999) to generate nearest neighbour designs for random allocation of herbicide (1.5 L/ha, 360 g/L glyphosate), insecticide (0.465 L/ha, 300 g/L dimethoate) and an untreated control. To ensure sufficient replication to generate neighbour balance, the experiment was designed on the assumption that the strips on each of the 2 sites contained plots that were contiguous.

Bi-directional sticky traps were used to measure insect movement from the neighbouring vegetation into the lucerne field and vice versa. Each trap (0.0637 m^2) was constructed from 10, 90-mm diameter petri dishes mounted on a wooden stake (1800 mm tall). The inner surface of each petri dish base was coated with a thin layer of Tanglefoot sticky trap glue (Australian Entomological Supplies, Bangalow, Australia). Five petri dishes (total area of 0.0318 m^2) faced the stand and the 5 plates on the opposite side of the stake faced the non-crop vegetation. The petri dishes were arranged vertically on each face of the stake, with their edges touching. The centre of the lowest dish was 300 mm from the soil surface and the centre of the top dish was 690 mm from the soil surface. Each smaller petri dish was nested within the lid of the petri dish, with its sticky surface outermost. Both were secured to the stake with a drawing pin. The petri dish lids had previously been sprayed with 3 coats of acrylic yellow paint (Carnival Yellow, Dulux, Clayton, Vic., Australia). The coloured lids remained attached to the stakes and the sticky bases were collected twice weekly, between 21 September 2001 and 5 November 2001. This period was the 45 days immediately following the application of treatments to the non-crop vegetation. For each plot, a single trap was placed near the boundary of the lucerne field/non-crop vegetation, 10 m from the plot's edges. For each collection date, leafhoppers on each trap were identified and counted using a binocular microscope (10×). Catches of each leafhopper species were pooled over all dates. Analysis of variance, using Genstat (Genstat Committee 2002), was used to test for effects of pesticide treatment, direction of flight and trap height, following a square root transformation [$\sqrt{(x + 0.5)}$] on all data.

Experiment 2. Four certified lucerne (cv. Aurora) seed stands were established in the mid Lachlan Valley and separated by a minimum of 10 km. These irrigated stands had adjacent vegetation consisting of *E. plantagineum*, *S. marianum*, *O. acanthium*, *Marrubium vulgare* L.

(horehound), *Cucumis myriocarpus* E. Mey. ex Naud. (paddy melon) and *Heliotropium europaeum* L. (heliotrope). At each site, a strip of non-crop vegetation, at least 200 m long and 10–20 m wide, was marked along the entire length of opposite boundaries of the field. This strip was chosen so that the vegetation within it was relatively homogenous for botanical composition and vigour. Each plot of non-crop vegetation was divided into 4 plots of at least 50 m in length. Each plot was assigned randomly to a different treatment: herbicide (1.5 L/ha, 360 g/L glyphosate); insecticide (0.465 L/ha, 300 g/L dimethoate); a combination of both insecticide and herbicide, at the above rates; or an untreated control. Treatments were reapplied 34 days after the initial application. A total of 8 replicate blocks was used (i.e. 2 blocks per site, 4 sites).

Insect movement, into and out of each lucerne stand, was monitored using bi-directional yellow sticky traps (total area of 0.0254 m^2) placed on the crop margin. Traps were constructed as described in experiment 1, using 2 petri dishes facing in each direction, the centre of the bottom dish being 300 mm from the soil surface and the centre of the top dish being 390 mm from the soil surface. Two traps were placed in the lucerne stand near the boundary with non-crop vegetation, 5 m either side of the mid-point of the plot. The traps were changed weekly (from 12 November 2002 to 23 December 2002) and, for each collection date, leafhoppers on each trap were identified and counted using a binocular microscope (10×). Catch data were pooled across all collection dates following a square root transformation [$\sqrt{(x + 0.5)}$]. Analysis of variance, using Genstat (Genstat Committee 2002), was used to test for effects of pesticide treatments, direction of flight and trap height for each leafhopper species.

In experiment 2, disease severity was assessed by delineating an arc with a radius of 30 m in the lucerne, adjacent to each border treatment plot, using a string attached to the midpoint of each plot's edge. This was done on 30 January 2003, when observations indicated the appearance of ALuY symptoms. Within each arc, counts were made of all symptomatic lucerne plants.

Results

Symptom and leafhopper survey

ALuY symptoms developed 29 weeks after sowing at site 1 and 31 weeks after sowing at sites 2 and 3. At all sites, maximum numbers of symptomatic plants on any given date were relatively low. Dates that had a symptomatic plant count maximum of less than 5 were excluded from the analyses. Separate 3-dimensional maps of symptomatic plant numbers, symptom severity and symptomatic plant density showed no significant differences among sample dates, so regression analysis was performed on symptomatic plant numbers only. Regression analysis of data from site 1 on 2 dates (4 January 2001 and 8 February 2001) indicated statistically significant spatial effects (Fig. 2). Regression models accounted for up to 20.6% of the variability among 300 ALuY symptomatic plants of about 10 000 plants sampled. On both dates, numbers of symptomatic plants were significantly higher in some parts of the crop margin than in other parts of the margin and tended to decrease with distance from 1 edge. On 4 January 2001 there was a peak away from the edge, but statistically the numbers decrease from 1 boundary to the opposite boundary.

Distribution of both *A. torrida* and *B. angustatus*, showed a significant edge effect at sites 1 and 3, on at least 1 date (Figs 3 and 4 respectively). Whenever a significant spatial effect was found, catches of leafhoppers were highest in 1 or more sections of the crop margin and lower in the stand interior, though catches were not consistently high in all margins. At site 1, symptomatic plant numbers on 8 February 2001 were positively correlated with the *O. argentatus* distribution observed 9 days earlier, on 31 January 2001 ($r = 0.195$, $P = 0.05$). Symptomatic plant numbers at site 3 showed a correlation on 20 February 2001 with the spatial distribution of *B. angustatus* 54 days earlier, on 28 December 2000 ($r = 0.318$, $P<0.05$). Similarly, there was a significant correlation between the symptomatic plant numbers on 4 January 2001 and the spatial distribution of *A. torrida* on 11 April 2001 at site 1 ($r = 0.300$, $P<0.05$).

Leafhopper species were found on plants adjacent to the monitored lucerne stands at all 3 sites. *Austroagallia torrida* was common on *M. vulgare*, *Polygonum aviculare* (Hogweed),

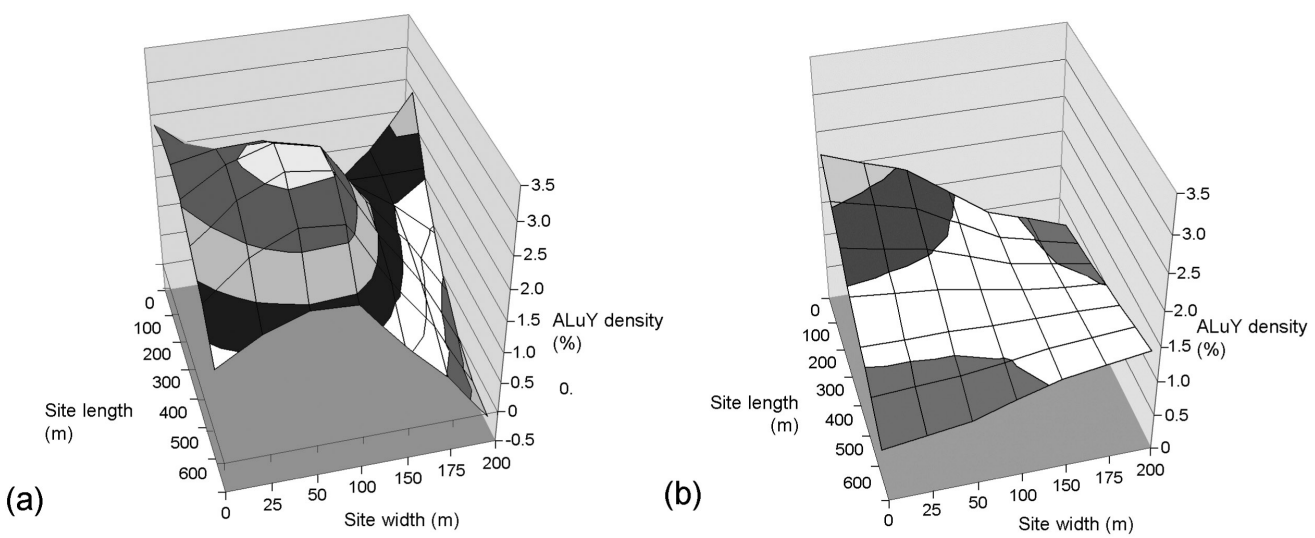

Figure 2. Fitted models representing spatial distribution of ALuY disease severity for site 1 on (*a*) 4 January 2001 and (*b*) 8 February 2001.

C. album and *H. europaeum*. *Orosius argentatus* was common on *H. europaeum*, *M. vulgare*, *P. aviculare* and *C. album*. *Batracomorphus angustatus* was less abundant than other leafhoppers, but most common on *P. aviculare*, *C. myriocarpus* and *H. europaeum*. Trends in the distribution of leafhoppers in non-crop vegetation were not detectable and were not correlated with symptomatic plant numbers within the stand.

Border treatment experiment

Experiment 1. Only 9 *B. angustatus* were caught in all treatments over the course of the experiment, so data for this species were excluded from the analyses. Border treatments did not significantly affect catches of either *A. torrida* and *O. argentatus*, but catches were strongly affected by trap height, with catches declining as trap height increased (Fig. 5*a* and *b*).

Experiment 2. No statistically significant spatial effects were found in numbers of *B. angustatus*. Irrigation at 1 of the sites ceased in early November 2002, due to the grower's reduced water allocations during drought conditions. This site was excluded from the analyses, as desiccation of the stand and non-crop vegetation led to low insect catches for all treatments at that site. For the remaining 3 sites, pooled counts of *A. torrida* and *O. argentatus* were significantly higher in the lower traps (Fig. 5*c* and *d*). Herbicide treatment reduced *A. torrida* migration into the lucerne, as well as overall (i.e. pooled immigration and emigration) catches, to a statistically significant ($P = 0.02$ and $P = 0.005$, respectively) extent, compared with the control treatment (Table 2). Similarly, catches of immigrating *O. argentatus* were reduced significantly by herbicide treatment (Table 2). Catches of

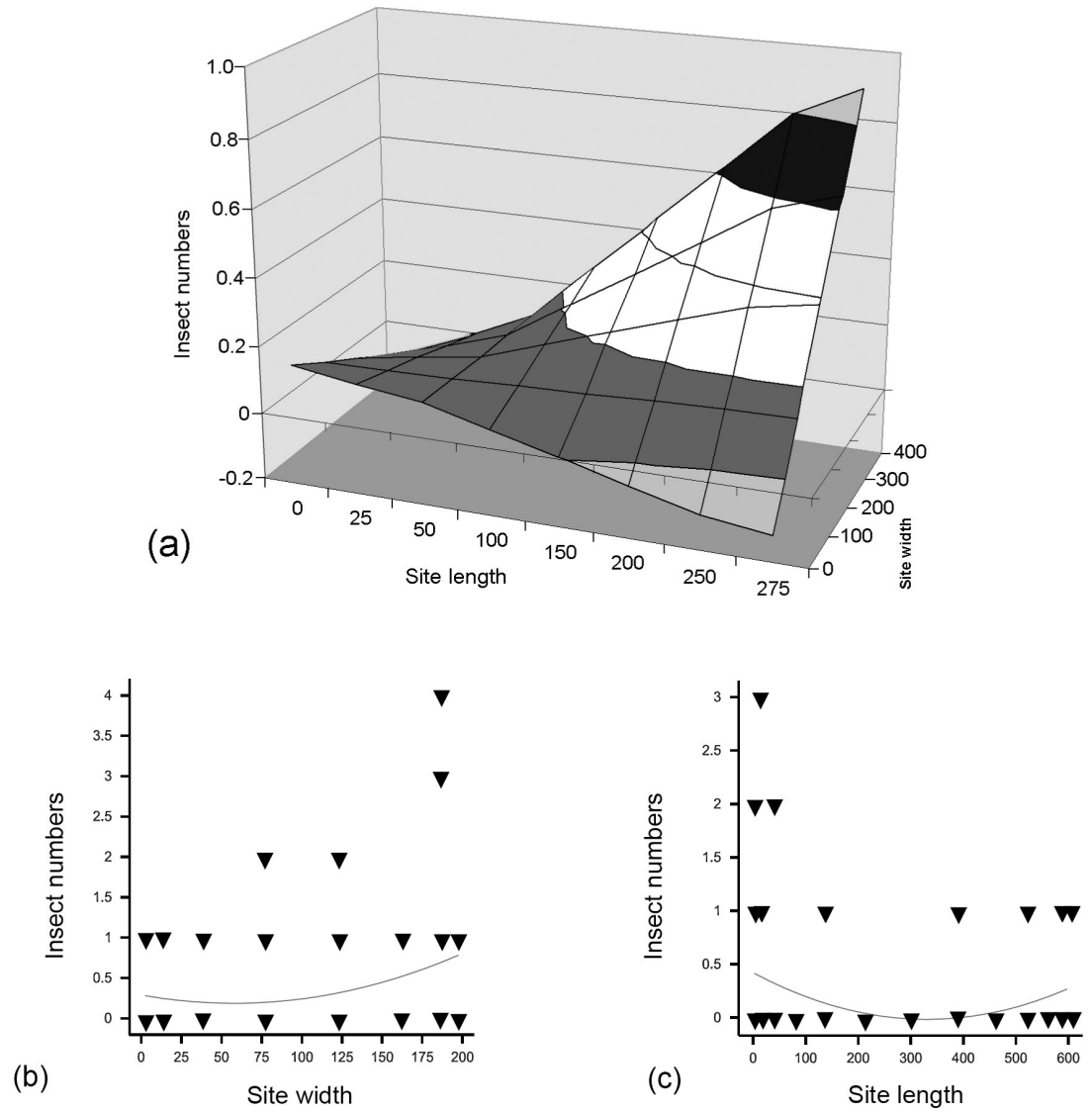

Figure 3. Fitted models representing spatial distribution of *B. angustatus*: (*a*) 3-dimensional distribution on 28 December 2000 at site 3; (*b*) 2-dimensional distribution on 20 November 2000 at site 1; and (*c*) 2-dimensional distribution on 29 December 2000 at site 1.

emigrating *O. argentatus* were reduced significantly by insecticide treatment. Throughout all treatments, there were higher numbers of *O. argentatus* and *B. angustatus* migrating into the stand than migrating away from the stand.

The numbers of plants expressing ALuY symptoms was significantly (P = 0.007) reduced (mean = 4.50) in plots treated with insecticide, when compared with the untreated control (mean = 6.33). The reduction in disease levels achieved by herbicide treatment (mean = 5.00) fell just outside of 95% confidence limits of significance (P = 0.052), when compared with the untreated control.

Discussion

At all 3 sites, ALuY symptoms appeared within 32 weeks of the stand being sown, showing that losses may be experienced even in the first season of seed stands. The period between inoculation and expression of symptoms known for other phytoplasma diseases is about 40–60 days in the case of the eastern peach X-mycoplasma-like organism (Chiykowski and Sinha 1988). It is as low as 16–25 days in other phytoplasma pathosystems (Chiykowski and Sinha 1990). The period between sowing and disease expression in the present study allows for the possibility of an insect vector of ALuY.

Leafhoppers are known vectors of several phytoplasma diseases (Davis and Sinclair 1998), such as sugarcane white leaf (Hanboonsong *et al*. 2002), aster yellows (Beanland *et al*. 1999) and European stone fruit yellows (Carraro *et al*. 2001). Reservoirs of the pathogen in crop-margin vegetation are suspected in other pathosystems to constitute a source of inoculum (Wilson *et al*. 2001) and may also be hosts to vectors (Lee *et al*. 2001). If such a scenario were indicated for ALuY, management of non-crop vegetation may reduce

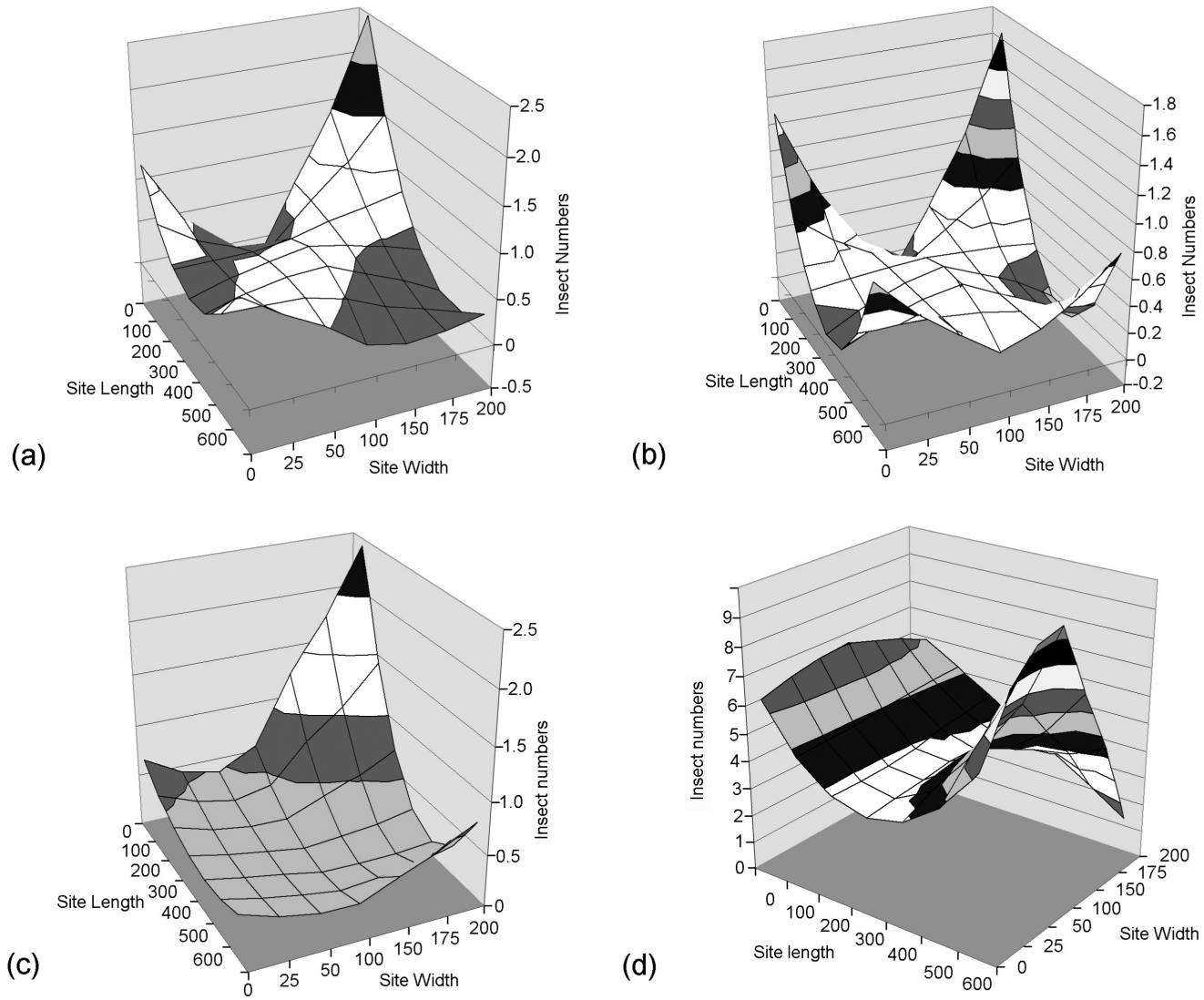

Figure 4. Fitted models representing insect numbers at site 1: (*a*) *A. torrida* on 29 December 2000; (*b*) *A. torrida* on 19 January 2001; (*c*) *A. torrida* on 31 January 2001; and (*d*) *A. torrida* on 13 February 2001.

disease severity by minimising the pathogen reservoir and/or limiting vector immigration, as observed for strawberry mottle virus by Raworth and Clements (1990).

The spatial distribution of leafhoppers on some dates was significantly correlated with symptomatic plant numbers on other dates. On 28 December 2000, the distribution of *B. angustatus* at site 3 was significantly correlated with the distribution of symptoms 54 days later. The length of this incubation period is consistent with that known for other phytoplasma diseases (Chiykowski and Sinha 1988). While the distribution of *O. argentatus* at site 1 was correlated with disease incidence only 9 days later, the immigration of the vectors into the stand may have happened up to 12 days earlier, when the preceding sample was taken. This places the disease incubation time within the range known for other phytoplasma diseases (Chiykowski and Sinha 1990). Catches of *A. torrida* before emergence of ALuY symptoms may have been too highly variable to allow the detection of a statistically significant relationship with symptoms, though a significant relationship between symptoms and later densities of *A. torrida* was found. Availability of the pathogen reservoir may have dictated that the vectoring of the disease into the lucerne crop occurred over a short period of time and not continually through the growing season. This may also explain the low level of correlation between symptom and leafhopper distribution.

Caution is required in interpreting correlations between insect catches and symptoms, because of the danger of a type

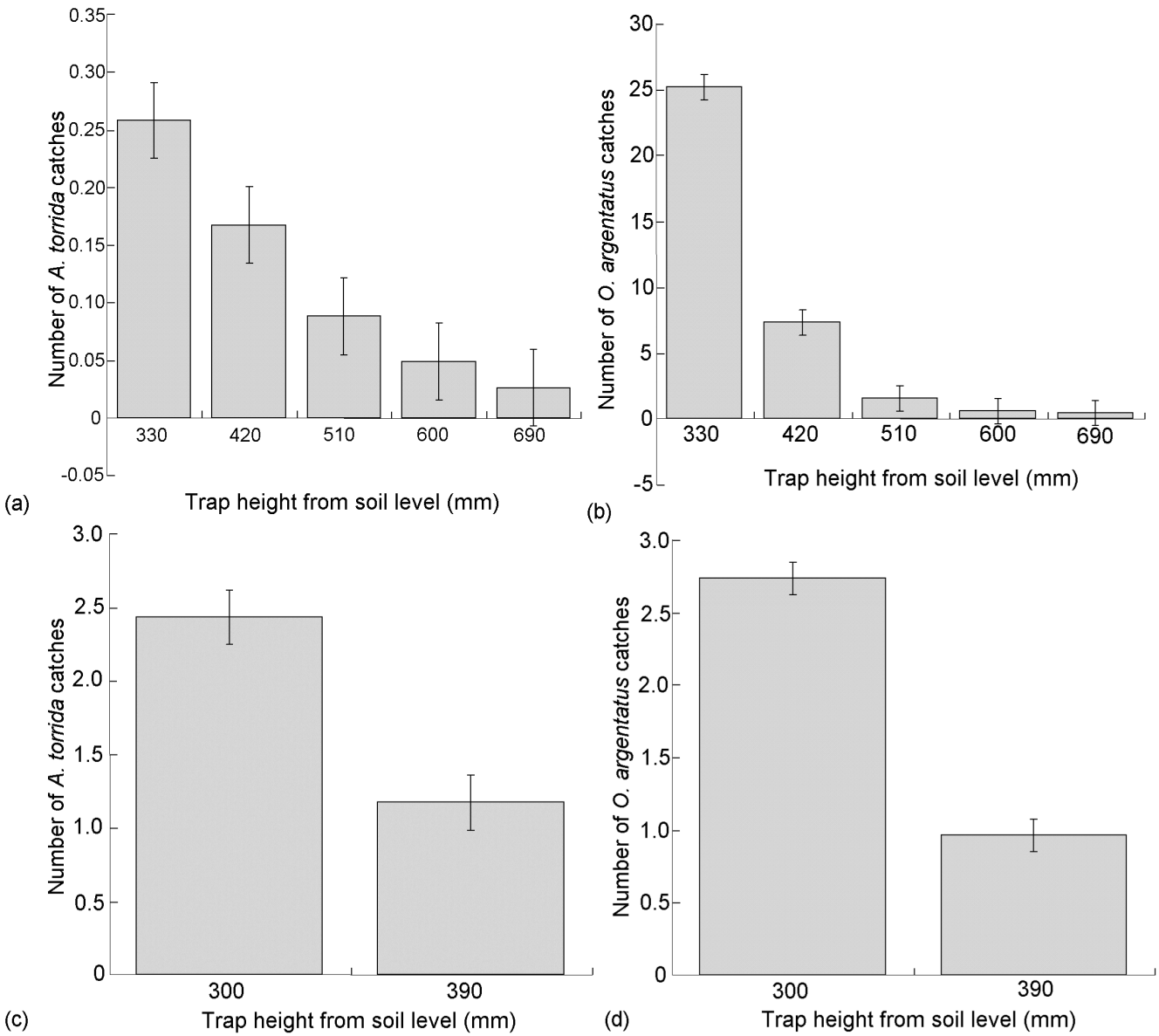

Figure 5. Effect of trap height on catches of *A. torrida* and *O. argentatus* in experiments 1 (*a* and *b*) and 2 (*c* and *d*).

I statistical error resulting from the large number of combinations that were used. Three significant correlations were detected from a total of 38 insect species–symptom relationships tested. Despite this risk, and the fact that correlations do not constitute evidence for causality, the spatial results are consistent with the hypothesis that *O. argentatus*, *B. angustatus* and, to a lesser extent, *A. torrida* are presumptive vectors for ALuY. Evidence from other experiments will be useful to further test this hypothesis for each species.

Significant edge effects were evident for *A. torrida* and *B. angustatus* on several dates. The spatial distribution for *A. torrida* on 13 February 2001 (Fig. 4*d*) differed to previous dates. It is possibly explained by higher numbers of leafhoppers resulting from a large migration event between sampling dates. This difference, and the detection of this difference, is indication that extensive sampling over a long period of time was warranted.

Table 2. Effect of border treatments on catches of leafhoppers on lucerne borders when compared with the control

Treatment and flight direction	Mean total catches	Comparison with control l.s.d.	*P*-value
Austroagallia torrida			
Herbicide and insecticide			
Immigration	1.25	0.201	0.223
Emigration	1.19	0.157	0.519
Pooled	1.22	0.127	0.176
Herbicide			
Immigration	1.15	0.190	0.020
Emigration	1.10	0.166	0.105
Pooled	1.12	0.126	0.005
Insecticide			
Immigration	1.26	0.211	0.311
Emigration	1.19	0.167	0.558
Pooled	1.23	0.135	0.247
Control			
Immigration	1.37		
Emigration	1.24		
Pooled	1.30		
Orosius argentatus			
Herbicide and insecticide			
Immigration	1.63	0.173	0.098
Emigration	1.33	0.135	0.378
Pooled	1.48	0.112	0.069
Herbicide			
Immigration	1.33	0.158	0.061
Emigration	1.10	0.166	0.105
Pooled	1.26	0.103	0.029
Insecticide			
Immigration	1.33	0.153	0.056
Emigration	1.13	0.119	0.024
Pooled	1.23	0.004	0.098
Control			
Immigration	1.48		
Emigration	1.26		
Pooled	1.37		

The significant edge effects, evident in field surveys for *A. torrida* and *B. angustatus*, are consistent with the finding that leafhopper catches were greater in lower traps than in identical traps placed further from the ground. This suggests that the leafhoppers do not undergo long-range dispersal to reach newly-sown lucerne stands, but enter by trivial, short-range movement from adjacent vegetation. There were no significant spatial patterns detected for the leafhopper *O. argentatus*, though casual observation and marginally non-significant patterns (data not shown) suggest the presence of higher numbers in the crop margins than in the stand interior.

In the present study, the use of herbicide reduced the overall catches of *A. torrida* and *O. argentatus* and the migration of *A. torrida* into the stand. Insecticide reduced the migration from the stand of *O. argentatus*. In experiment 2, reduced leafhopper movement was associated with a reduction in the numbers of symptomatic plants adjacent to the plots treated with insecticide. There are several possible reasons for this evident reduction in migration of these leafhoppers. Evidence from the height analysis of the sticky trap catch data suggests that these leafhoppers move over short distances with low-level flight. The removal of appropriate vegetation may remove their ‘corridor’ into the lucerne stand. By removing their ability to colonise non-crop vegetation adjacent to a crop susceptible to ALuY disease, the leafhoppers may migrate to other suitable host plants nearby but will be unable to travel into the lucerne crop. This will reduce the levels of ALuY introduction into the crop.

Taken with the spatial trends and correlations, this suggests that *A. torrida* and *O. argentatus* are presumptive vectors of ALuY, though transmission tests or molecular studies are required to verify this and the possibility that *B. angustatus* is a vector cannot be ruled out. The result also suggests that disease management strategies which minimise immigration of leafhopper species from non-crop vegetation into the stand may be successful on a larger scale.

In this study, most leafhoppers were caught in low sticky traps, which suggests that the their dispersal is short-range, and ALuY disease incidence was lower adjacent to insecticide treatments. It is, therefore, likely that the initial immigration of leafhoppers is an important event in the ALuY pathosystem. Further, the observation of reduced ALuY disease incidence was 10 weeks after the application of border treatments. This suggests that immigration of infective insects may be more important than within-crop movement of insects, including the progeny of immigrants. Accordingly, a broad-scale reduction in the initial movement of leafhoppers into the crop could reduce losses caused by ALuY disease.

The use of an ALuY management strategy based on heavy pesticide inputs to large areas of non-crop vegetation is unlikely to be acceptable to farmers, regulatory authorities or the broader community. A narrow strip of treated non-crop

vegetation, however, may suffice to give useful levels of disease suppression. A normal application rate would require 4.9 kg of dimethoate to treat a 35 ha stand of lucerne, in comparison with 660 g of dimethoate to treat a 20 m wide band of the non-crop vegetation bordering the stand. It would seem that removal of host species with an effective weed control strategy might have the most potential and greatest acceptability to farmers.

Further work may show that pesticides are not required in management strategies developed for ALuY. During the study by Schaber *et al*. (1990), it was seen that physical barriers, such as farm access roads or irrigation channels, limited the movement of all insects with a short flight pattern similar to the leafhoppers examined in this study. Given that the results from this study suggest that the leafhoppers are moving only short distances, physical structures, such as shade-mesh barriers mounted on existing fences, may provide an adequate barrier against vector immigration.

Results indicate that the successful management of ALuY disease may be achieved by limiting the movement of leafhoppers into lucerne stands, though further studies need to be undertaken. The reduction of symptomatic plants in relation to the lowered movement of *O. argentatus* and *A. torrida* into the lucerne stand is the best indication, to date, that one or both of these leafhopper species is a vector of ALuY.

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