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An analysis of fruitspotting bug activity in avocado crops from fruit-set to harvest

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Summary

The banana-spotting bug, *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae), is a major insect pest of avocados and other horticultural fruit crops of economic importance in Queensland, Australia. Despite the severity of the pest, very little is known about its ecology or behaviour in horticultural orchards. To better understand the relationships between the pest and its avocado host plants, studies investigated the survival, development and performance of bugs on avocado and two other important horticultural crop hosts, papaya and lime. Recent advances in the understanding of the chemical ecology of banana-spotting bugs have led to the development of pheromone traps for the pest that are attractive to nymphs and both sexes of adult. These pheromone traps were used to investigate the seasonal abundance and spatial distributions of banana-spotting bug populations in avocado crops. The efficiency of the traps for catching bugs at different stages of development was also studied in the field and the utility of traps for concentrating bug populations for possible control with targeted applications of insecticide was also investigated.

The different phenological stages (flowers, vegetative flush and different sized fruit) of avocado (*Persea americana* Mill. cv. Shepard), lime (*Citrus aurantifolia* L. cv. Tahitian) and papaya (*Carica papaya* L. cv. Hybrid 1B) crops were evaluated for their suitability as hosts for banana-spotting bug. When fed exclusively on a given stage of a given host plant, bugs could only complete development to adults when fed vegetative flush of papaya, papaya flowers, lime flowers or green bean pods. The latter were included as controls as they are known to be suitable for banana-spotting bug growth and development. Avocado was not a suitable host plant and nymphs did not survive beyond the second instar when provided with any avocado plant parts (including small, medium and large fruit) alone. Further, nymphs did not survive beyond the second instar on small, medium or large lime or papaya fruit. On plant parts on which development could be completed, mean nymph- adult developmental times ranged from 40 days (on papaya flush) to 59 days (on lime flush). Although a small number of banana-spotting bugs did complete development on lime flush, none of the adult females that developed subsequently laid eggs. The pre-oviposition period, weight and fecundity of adult females were also affected by host plant and stage; female banana-spotting bugs developing on papaya flush and green beans had shorter pre-oviposition periods and were heavier and more fecund than those developing on papaya flowers.

Field trials in a mango orchard evaluated the sampling bias of pheromone traps by comparing the composition of trap catches to the banana-spotting bug population structure in the orchard as estimated by insecticide knock-down of all insects in the canopy of selected trees. Significantly more adult females than adult males and significantly more adults than nymphs were caught in pheromone traps. Insecticide knock-down showed that mango trees containing pheromone traps harboured more female banana-spotting bugs than trees that did not contain pheromone traps.

Long term (2 year) pheromone trapping of banana-spotting bugs in avocado crops recorded the highest population densities between October and May (peak densities in early May) and the lowest densities between July and September. Significant adult population aggregations were identified on an avocado block next to a lime block during both years of the study. Significant nymph population aggregations were also identified on an avocado block next to native riparian vegetation. Spatial analysis from pheromone trap data (collected over a month) identified significant population aggregations in the lime block and spatial gaps in the avocado block.

Field trials examined the relationships between banana-spotting bug feeding damage and the number of bugs captured in pheromone traps on two avocado blocks. Feeding damage on avocado fruit in trees containing pheromone traps was higher than that in trees without pheromone traps. Positive correlations were detected between feeding damage and banana-spotting bug numbers captured in pheromone traps within a given tree. In trees containing pheromone traps, a significantly higher proportion of avocado fruit sustained feeding damage than the proportion of fruit that was damaged in trees 6 m and 18 m from pheromone traps. Significantly more feeding sites per avocado fruit were recorded in fruit from trees containing pheromone traps compared to trees without traps.

The implications of this research for developing improved pest management strategies that integrate pheromone traps to monitor and manipulate banana-spotting bug populations are discussed.

Keywords

Amblypelta lutescens lutescens, avocado, banana-spotting bug, behavior, ecology, host plants, integrated pest management, lime, papaya, pheromone trap.

Introduction

The banana-spotting bug, *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) is an important insect pest of avocados in Queensland, Australia (Waite et al. 2000). Economic damage is caused by a combination of the costs of banana-spotting bug control, damage to fruit that renders it unmarketable and premature fruit drop. Feeding damage to avocado fruit occurs from fruit-set to harvest (October–March) (Waite et al. 2000). Current pest management strategies for *A. l. lutescens* in avocado orchards in Far North Queensland involve regular calendar based applications of broad-spectrum synthetic pyrethroid and organophosphate insecticides every 10-14 days when avocado fruit are on the trees (October–March). The application rates often exceed the number of recommended applications on the relevant insecticide labels. Excessive broad-spectrum insecticide applications disrupt beneficial invertebrates such as predators, parasitoids and pollinators and prevent the use and development of Integrated Pest Management (IPM) strategies. The main components of the pheromone emitted by male *A. l. lutescens* have been identified (Aldrich et al. 1993, Khrimian et al. 2012) and a pheromone trap that uses components of the pheromone as a synthetic attractant has been developed. Traps baited with the lure capture adult males, females and immature nymphs (Khrimian et al. 2012). The pheromone trap has created new opportunities to develop alternative pest management strategies for the pest.

The overall objective of this study was to improve understanding of the ecology and behaviour of *A. l. lutescens* in relation to avocado crops and to investigate the potential of using the pheromone traps to improve pest management. The relative suitability of avocado, lime and papaya crops as host plants for *A. l. lutescens* was examined by assessing the survival, development, pre-oviposition period and fecundity of *A. l. lutescens* reared on the different crops. Different plant structures that included vegetative flush, flowers, and fruit (at different stages of development) and which represented different phenological stages of the crops were investigated. The suitability of green beans as a food source was also examined as they have previously been reported to be a good food source for *A. l. lutescens* (Waite *et al.*, 2000) and they were in included in experiments, as appropriate, as positive controls. The potential sampling bias of *A. l. lutescens* pheromone traps and their efficiency at trapping *A. l. lutescens* of different ages and sexes was assessed by comparing trap catches with the age structure of the field population of *A. l. lutescens* in a mango orchard. The age structure of the field population was estimated by insecticide knock-down of insects in selected trees. Adult female, male and immature nymph catches were then compared between the two sampling methods. Further, the age-structures of the *A. l. lutescens* populations in mango trees with and without pheromone traps were also compared by the insecticide knock-down method. Laboratory experiments examined the pheromone trap capture rates of different *A. l. lutescens* life stages over a 12 h period. A two-year field study investigated *A. l. lutescens* population densities over time and spatial distributions using weekly pheromone trap data on two independent avocado blocks. The pheromone traps were arranged in a uniform grid (36 m x 36 m) to investigate spatial patterns in relation to the proximity of native riparian vegetation and other horticultural crops. The possible utility of employing pheromone traps for improved pest management was examined in a study that explored correlations between *A. l. lutescens*-damaged avocado fruit and the number of *A. l. lutescens* captured in pheromone traps. The potential of an "attract and kill" pest management strategy was investigated by correlating avocado fruit damage in trees baited with pheromone traps with pheromone trap catches in those trees and comparing avocado fruit damage in trees baited with pheromone traps with fruit damage in trees in trees 6 m and 18 m away.

Methodology

Development, survival and fecundity of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) on distinct phenologcal stages of different crop host plants (Appendix 1).

Experiments investigated survivorship and development of *A. l. lutescens* on different phenological stages of avocado, lime, papaya and green bean host plants under controlled environmental conditions (27±0.5 °C; 70±10% RH; 14: 10 L: D photoperiod). Prior to each experiment, eggs were collected daily from 20-30 pairs of adult male and female *A. l. lutescens* that were maintained in culture. Single newly hatched neonate nymphs (<24 h old) were distributed equally among the treatments and individual insects were held with their designated food sources in ventilated 250 ml containers. Depending on the availability of nymphs, approximately 30 neonates were reared separately on each plant part. Three replicates (n≈30 neonates per replicate) were prepared for each plant part treatment. Flowers and vegetative flush material were replaced every second day while fruit were replaced every five days. Insects were checked daily and mortality and stage of development recorded. When insects completed the final molt they were individually weighed and their weight recorded (<24 h after molt). Male and female insects that had completed pre-imaginal development on the same food source were then paired in ventilated 250 ml containers, supplied with the same plant part of the crop on which they had developed and incubated further (27±0.5 °C; 70±10% RH; 14: 10 L: D photoperiod). Insects and plant parts were then checked daily. The number of eggs laid by each female was recorded each day and each pair of insects was monitored for 21 days following the first oviposition event.

Sampling efficiency and bias of pheromone traps capturing *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) (Appendix 2).

To test for potential pheromone trap sampling bias, 12 pheromone traps were distributed in selected rows across a mango orchard; traps were spaced 25-60 m apart. Pheromone traps consisted of green twin-walled polypropylene sheeting (Corflute®) covered by double sided tape coated with an acrylic adhesive. A thin wire was used to secure the rubber septa with the pheromone in a hole at the centre of the trap. Pheromone trap catches were examined weekly for 22 weeks from 30/10/2014 – 26/03/2015 and the number of adult male, female, and nymphs of each instar captured in each trap each week was recorded.

Insecticide knock-down sampling of canopy dwelling arthropods was conducted on the same mango block on five occasions using the synthetic pyrethroid *β*-cyfluthrin (Bulldock®, Bayer). The insecticide was applied using either an airblast sprayer behind a tractor or using a Solo® 10 L knapsack sprayer. Three to five mango trees with pheromone traps ("trap" trees) and three to five mango trees without pheromone traps ("non-trap" trees) were sampled per session. Prior to application of insecticides, blue tarpaulins (6 m x 3 m) were positioned under "trap" and "non-trap" mango trees. Approximately one hour after insecticide applications, "trap" trees and "non-trap" trees were shaken vigorously for one minute and then the tarpaulins were inspected for ten minutes. All banana-spotting bugs (nymphs and adult males and females) that dropped onto the tarpaulins were collected and recorded.

Two insect cages (3m x 2m x 2m) were used to investigate the responses of different *A. l. lutescens* life stages (nymphs of each instar, adult male and adult female bugs) to the pheromone trap under glass house conditions. One cage was used as a treatment cage and contained a pheromone trap

with the attractant lure, while the other cage was used as a control cage and contained a trap without the attractant lure. Two avocado seedlings were placed in the middle of each cage and the pheromone trap was attached to the frame of the cage between the avocado seedlings. At the start of each experiment 16 adult males, 16 adult females and 16 nymphs of each instar were released into the cages. Approximately 24 hours later, at 06:00, the pheromone and control traps were introduced into the cages where they remained for 12 h. Traps in both treatment and control cages were checked every hour during the 12 h period and any adult or nymph *A. l. lutescens* captured were recorded and removed. Traps were removed after 12 h. The experiment was repeated on three separate occasions.

Population monitoring and spatial distributions of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) in avocado crops (Appendix 3)

Adult and nymph *A. l. lutescens* populations in two avocado orchards (designated orchard A and orchard B), and a lime orchard adjacent to avocado orchard A in Muchilba Queensland, were monitored using pheromone traps. Populations in the avocado orchards were monitored over a two-year period while the population in the lime orchard was monitored over a four-week period during December 2013. Pheromone traps were arranged in a 36 m x 36 m grid across the avocado and lime orchards. Each pheromone trap was examined weekly and any adult males, adult females, and nymphs captured in traps were recorded. Spatial distributions were investigated using the Spatial Analysis by Distance Indices (SADIE) program. (SADIEShell, version 2.0,home.cogeco.ca/_sadiespatial/SADIEShell.html) (Perry 1995, Perry et al. 1999). In the avocado orchards, SADIE spatial analyses were conducted on weekly pheromone trap catches, on year-end cumulative pheromone trap catch data for each year of the study and on total pheromone trap catches from the lime crop and the avocado orchard A during December 2013.

Potential applications of pheromone traps for IPM of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) in avocado crops (Appendix 4).

On two avocado orchards, the relationship between avocado fruit with *A. l. lutescens* feeding damage and *A. l. lutescens* captured in pheromone traps was examined by conducting detailed assessments of fruit damage every two weeks. Damage assessments were conducted on 10 randomly tagged avocado fruit on avocado trees containing pheromone traps, avocado trees that were 6 m from trees containing traps (neighbor trees) and trees 18 m from trees containing tarps (distant trees). At sampling point new feeding sites were recorded and marked with a permanent marker to prevent them being counted again in future damage assessments. *Amblypelta l. lutescens* adults and nymphs captured in pheromone traps during the period were recorded at the same time as damage assessments and insects were removed from the traps. At the end of the field trial, the potential of an attract and kill pest management strategy was investigated by harvesting tagged avocado fruit from all experimental trees at each field site. The skin of each fruit was peeled to determine the relationship between feeding sites visible on the skin and the actual number of feeding sites that penetrated the flesh of each fruit. Data were then analysed to determine to whether avocado fruit in trees containing pheromone traps sustained more damage than fruit in trees without pheromone traps that were located 6 and 18 m away.

Outputs

New knowledge and technology

- Avocado is a poor host for *A. l. lutescens*

- First instar *A. l. lutescens* were observed feeding and survival rates were affected by the quality of food provided.

- In avocado crops in far North Queensland *A. l. lutescens* densities are typically high from October through to June and lower from July to September. *Amblypelta l. lutescens* population densities are not correlated with the presence of fruit on trees and high densities of the pest can remain in avocado orchards after harvest.

- Spatial analysis of *A. l. lutescens* distributions in avocado crops shows that population densities are high in areas close to lime crops, suggesting that lime crops might generate source populations that invade avocado crops

- When pheromone traps are placed in avocado trees the density of *A. l. lutescens* caught in traps is positively correlated with fruit damage in the tree. Pheromone traps can concentrate *A. l. lutescens* populations in trees, providing a specific target for reduced input insecticide control strategies

Articles/ publications

Conference abstracts:

Lindsay, K. R., Zalucki, M. P., Newton, I. R., Furlong. M.J. An Analysis of Banana-Spotting Bug Activity in Avocado Crops from Fruit-Set to Harvest. International Chemical Ecology Conference. 19-23 August 2013, Melbourne, Australia

Lindsay, K. R., Zalucki, M. P., Newton, I. R., Furlong. M.J. Effect of host plants on development, survival and fecundity for *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae). International Horticultural Congress. 17-22 August 2014, Brisbane, Australia

Lindsay, K. R., Zalucki, M. P., Newton, I. R., Furlong. M.J. 'Spatial and temporal dynamics of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) in avocado crops' Australian Entomological Society Conference. 28 September – 1 October 2015. Cairns, Australia

Lindsay, K. R., Zalucki, M. P., Newton, I. R., Furlong. M.J. 'Spatial and temporal dynamics of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) in avocado crops' Entomological Society of America Conference. 15-18 November 2015, Minneapolis, United States of America

Journal article (Submitted for publication March 2016):

Lindsay, K. R and Furlong, M. J. Development, survival and fecundity of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) on distinct phenological stages of different crop host plants. Journal of Economic Entomology.

PhD Thesis (to be submitted May 2016)

Lindsay, K. R. The ecology and behavior of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) in avocado crops. PhD thesis. The University of Queensland, St Lucia, Brisbane

Outcomes

Development, survival and fecundity of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) on distinct phenologcal stages of different crop host plants (Appendix 1)

- The suitability of different plant structures of avocado, lime and papaya crops and green bean pods as host plants for *A. l. lutescens* is described based on the development and survival rates of nymphs feeding on each structure.

- Nymphs could not complete development on lime flowers, avocado flush or avocado flowers. Nymph survival to adulthood was high on papaya vegetative flush, papaya flowers and on green beans, while lower numbers completed development on lime flush (Table 1.1).

- Fruit of papaya, lime and avocado crops were not suitable for *A. l. lutescens* survival and development. No nymphs survived beyond the second instar on any of the fruits tested (Table 1.1).

- First instar nymphs were observed feeding. Their survival on vegetative flush and flowers was significantly greater than that on fruit of all host plants tested (Table 1.1). Contrary to previous claims, this indicates that the quality of food available to first instar nymphs affects their survival and that feeding in the first instar is important for survival in this species.

- When *A. l. lutescens* development was completed, the mean developmental time from egg hatch to adulthood ranged from 40 days on papaya flush to 59 days on lime flush (Table 1.2).

- When developing on papaya vegetative flush, papaya flowers or on green beans, adult females were heavier than adult males. The mean female pre-oviposition period ranged from 13 days on papaya flush to 32 days on papaya flowers and the mean number of eggs laid over a 21 day period (±SE) ranged from 5 (±0.5) eggs per female on papaya flowers to 48 eggs (±6) per female on green beans (Table 1.3).

Sampling efficiency and bias of pheromone traps capturing *Amblypelta lutescens lutescens* (Hemiptera: Coreidae) (Appendix 2).

- Insecticide knock down sampling of canopy dwelling arthropods in mango trees showed that pheromone traps catch more *A. l. lutescens* females than males and more adults than nymphs from the field population.

- Pheromone traps increase the density of female *A. l. lutescens* in fruit trees relative to trees without pheromone traps fruit trees

- In cage studies, capture rates of nymphs and adults of both sexes can be extremely low.

Population monitoring and spatial distributions of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) in avocado crops. (Appendix 3)

- Detailed pheromone trap data over a two-year period describes fluctuations in *A. l. lutescens* population densities and locations of population aggregations within avocado orchards. Pheromone trap data collected in an avocado block and lime block during December 2013 describes spatial distributions between the two crops.

- High population densities were recorded on avocado orchards from October - May, very low population densities occurred from July – September. Peaks in population densities were observed in early May and densities of *A. l. lutescens* were not affected by the harvest of avocado fruit.

- Population aggregations and high numbers of *A. l. lutescens* captured in pheromone traps were recorded on avocado orchards next to a lime crop and riparian forest.

Potential applications of pheromone traps for IPM management of *Amblypelta lutescens lutescens* (Hemiptera: Coreidae) in avocado crops (Appendix 4).

- The utility of pheromone traps in decision making to predict economic damage to avocado fruit and to act as the basis for an attract and kill pest management strategy were assessed.

- *Amblypelta l. lutescens* catches in pheromone traps can predict economic damage to fruit in the avocado trees in which traps are placed. Predictions are more reliable in smaller trees when fruit are closer to the pheromone trap.

- Only a small percentage (21-26%) of the total number of *A. l. lutescens* feeding sites on avocado fruit (as determined by detailed fruit dissection) was visible on the surface of the fruit.

- More *A. l. lutescens* feeding damage occurred on avocado fruit in trees with pheromone traps than on avocado fruit in adjacent trees that did not contain pheromone traps.

The host plant suitability of avocado, lime and papaya crops was established under controlled experimental conditions. Papaya was the most suitable host plant for *A l. lutescens* tested; on both flowers and vegetative flush a large proportion of nymphs survived to adulthood and female fecundity was high (Tables 1.1 and 1.3). Green bean pods were also suitable structures for *A l. lutescens* growth and development, a high proportion of nymphs developed to adults and adult female were very fecund when reared on green bean pods (Table 1.3). Lime was a less good host and *A. l. lutescens* survival to adult was lower, and development times of survivors significantly longer, than on papaya and green beans (Tables 1.1 and 1.2). No nymphs survived to adulthood on lime flowers. Avocado was not a suitable host and nymphs did not survive beyond the second instar on any phenological stages of this crop (Table 1.1). Avocado, lime and papaya fruit were not suitable for neonate nymph development to imago and no nymphs surviving past the second instar on any of the fruit tested (Table 1.1).

The sampling bias of pheromone traps was established. Compared to insecticide knock down sampling that censused the age structure of the field population of *A. l. lutescens* in a mango orchard, pheromone traps caught significantly more adult females than adult males (Table 2.1). Similarly, insecticide knock down showed that there were more adult females in mango trees with

pheromone traps than in trees without pheromone traps (Fig 2.1). Pheromone traps also caught more adults relative to nymphs (Table 2.1). Differences in mobility are a possible reason for this. Flightless nymphs are likely to experience difficulty reaching a pheromone trap fruit trees, as they have to follow the odour source by walking on branches. Results suggest that nymph catches in pheromone traps underestimate the density of nymphs relative to highly mobile adults in the filed. Cage experiments investigated pheromone trap capture rates of adult and nymph *A. l. lutescens* over a 12-hour period. Only low numbers of *A. l. lutescens* of all developmental stages were captured in pheromone traps in both treatment and control traps and no statistical differences were detected between the catches in pheromone baited and control traps (Table 2.2). The low numbers caught in traps in cage experiments could reflect the experimental conditions and it is also possible that pheromones released by the high densities of adult male *A. l. lutescens* released in cage experiments could have interfered with the efficiency of the traps baited with the synthetic lure.

Pheromone traps were successfully used to monitor *A. l. lutescens* field populations over a two-year period on two avocado blocks. During both years of the study, high *A. l. lutescens* population densities were recorded from October-May and lower *A. l. lutescens* population densities were recorded from July-September (Figure 3.3). These high population densities were not correlated with avocado fruit as high *A. l. lutescens* population densities were recorded after avocado fruit were harvested (late February or early March). Variations in population densities during the year are likely related to climate conditions as temperatures cooler than 25°C are detrimental to nymph survival (Govender 2015). Sudden increases in *A. l. lutescens* populations captured in pheromone traps were observed after events of heavy rainfall. Pheromone trap catch data and spatial distribution models identified significant population aggregations and high population densities occurring on an avocado block next to a lime crop (Figures 3.5 and 3.7). In addition, spatial distribution models identified population aggregations in the lime crop and spatial gaps in the adjacent avocado crop (Figure 3.6). These results strongly suggest that the lime crop could be a source of *A. l. lutescens* adults that move into the avocado orchard. Lime crops are a host plant for *A. l. lutescens* and were not managed with insecticides during the study. The grower considered *A. l. lutescens* to be a minor pest in the lime crop. Pheromone trap catch data and spatial distribution models also detected population aggregations of nymphs occurring on an avocado block near native riparian vegetation during the first year of the study (Figures 3.5 and 3.7). Native vegetation species near horticultural orchards are presumed to be the source of *A. l. lutescens* moving into horticultural orchards (Ryan 1994, Waite et al. 2000). However, there is still little understanding of host plant relationships between *A. l. lutescens* and native species due to difficulties in sampling the pest in this environment.

The potential use of pheromone traps for improved pest management strategies was successfully examined and provided insights into how pheromone could be utilized in the future. There was a significant correlation between *A. l. lutescens* feeding damage and *A. l. lutescens* captured in pheromone traps on small (<2 years old) avocado trees with pheromone traps compared to nearby trees without traps. However, correlations were not detected in large mature avocado trees with pheromone traps or in small and mature avocado trees without pheromone traps. Therefore, the reliability of predicted damage based on pheromone trap catch may be limited by distance from the pheromone trap. The study found that only a small proportion of the total *A. l. lutescens* feeding sites (21-26%) on fruit were externally visible while assessing feeding damage. Therefore, feeding sites counted during external damage assessments represent a significant underestimate of total feeding sites in avocado fruit. Fruit on avocado trees containing pheromone traps sustained more *A. l. lutescens* feeding damage than trees without pheromone traps on a commercial orchard. The effect of pheromone traps on fruit damage does not extend to neighbouring trees as these trees sustained significantly less feeding damage.

Evaluation and Discussion

Currently, pest management of *A. l. lutescens* on avocado orchards involves calendar-based applications of broad-spectrum insecticides every 10-14 days. The application rates exceed insecticide label recommendations and likely have an impact on beneficial invertebrates including predators, parasitoids and pollinators. Studies examined relationships between *A. l. lutescens* and avocado crops and the use of pheromone traps for population sampling and pest management. The research findings in this project provide avocado producers in Australia with significant new information on the ecology of *A. l. lutescens* principally: i) neonate nymphs feed on crops and their survival is influenced by the quality of food available, ii) avocado is not a good host for *A. l. lutescens* survival and development, iii) pheromone traps are more attractive to females than to males, iv) the number of adult *A. l. lutescens* caught in a trap correlates with fruit damage in the tree containing the trap and v) pheromone traps can be used to concentrate adult *A. l. lutescens* densities in avocado trees. The use of pheromone traps to sample for *A. l. lutescens* can play a significant role in development an IPM program, the initial phase of which could be based around the use of pheromone traps to concentrate adult *A. l. lutescens* populations in allowing the targeted use of insecticides, reducing overall insecticide inputs.

The research showed that horticultural crops such as limes grown near avocados could have an impact on avocado fruit damage. Adult *A. l. lutescens* populations in avocado crops during field trials are unlikely to have originated the crop due to a combination of regular insecticide applications and low avocado host plant suitability (Appendix 1). Spatial distribution analysis found significant clustering of *A. l. lutescens* populations on an avocado orchard next to a lime block that was not managed with insecticides during the study (Appendix 3). Lime crops were found to be better hosts for *A. l. lutescens* than avocado although limes were themselves poorer hosts than papaya (Appendix 1). It is possible that when *A. l. lutescens* populations in lime crops are not treated with insecticides that they can build up to high densities and provide a source population that can invade nearby avocado crops. The source of the *A. l. lutescens* populations that move into avocado orchards needs to be addressed when managing the pest. An area wide management approach that suppresses *A. l. lutescens* populations in the locality is likely to be a more effective long-term approach to management than simply trying to manage *A. l. lutescens* populations on an orchard by orchard basis.

When *A. l. lutescens* pheromone traps are commercially available to avocado growers, they can be used to monitor population densities over time. High population densities were identified on two avocado blocks from October – May and low population densities were identified from July – September (Appendix 3). Pheromone traps should be placed in trees in late September in order to detect population increases and provide information required for informed decision making about when to apply insecticides. Pheromone traps will also provide information on the effectiveness of insecticides at reducing population densities and allow growers to make informed decisions on when to next apply insecticide and whether or not the product that they are using is effectively reducing pest densities. The occurrence of high population densities until May (Appendix 3) means avocado growers will need to continue monitoring populations and applying insecticides as required until the fruit is harvested. Pheromone traps will need to be distributed throughout avocado blocks to determine areas within the crop with high population densities. The distribution and monitoring of pheromone traps should be greatest in avocado crops that border other horticultural crops, as these areas are vulnerable to adult *A. l. lutescens* moving between crops. If high numbers of *A. l. lutescens* are captured near other horticultural crops insecticides should be applied to the neighbouring crops to

reduce overall population densities.

Correlations between *A. l. lutescens* feeding damage on avocado fruit and *A. l. lutescens* captured in pheromone traps indicate the potential use of pheromone traps for predicting economic damage to avocado fruit on trees (Appendix 4). Avocado producers will be able to make informed decisions on whether insecticide applications are necessary based on the number of *A. l. lutescens* captured in pheromone traps. The current study found correlations, but only in avocado trees containing pheromone traps. Therefore the reliability of predictions diminishes with distance from the trap. If avocado growers want to use the pheromone trap for predicting damage, the next step is to determine what the levels of fruit damage avocado growers are willing to tolerate before applying insecticide applications.

During the study, significantly more *A. l. lutescens* feeding damage was observed on fruit in avocado trees containing pheromone traps, indicating that *A. l. lutescens* densities are concentrated in trees containing pheromone traps (Appendix 4). A possible explanation for this is that the pheromone lure may well function as an aggregation pheromone. Such compounds typically cause insects to aggregate near the source of the pheromone rather than attract insects directly to the point source of the pheromone as sex pheromones do (Čokl and Millar 2009). Thus, pheromone traps on their own are not likely to be suitable for managing *A. l. lutescens* populations. However, they may have great utility as part of an attract-and-kill strategy whereby pheromone lures are deployed to attract and concentrate *A. l. lutescens* into specific trees or rows of trees which can then be treated with insecticide (El-Sayed et al. 2009). Further field trials are needed to evaluate the use of using pheromone traps for predicting economic damage and for an attract and kill strategy to reduce *A. l. lutescens* damage; these approaches will only be successful if they reduce *A. l. lutescens* damage and/or they reduce the quantity of insecticide used without compromising yield.

Recommendations

Recommendations for pest management

- Distribute pheromone traps on avocado orchards as a monitoring tool to identify areas with high population densities and to monitor changes in population densities over time.
- Begin monitoring for *A. l. lutescens* in October and continue monitoring until the avocado crop has been harvested.
- Apply insecticides to areas/ trees where high numbers of *A. l. lutescens* are captured in pheromone traps and when population densities begin to increase.
- Focus monitoring and insecticide applications in areas that are adjacent to other crops favourable for *A. l. lutescens* development including citrus, mango and papaya.
- Monitor *A. l. lutescens* populations in other horticultural crops near avocado crops and apply insecticides to these crops when numbers reach high levels to reduce movement of *A. l. lutescens* into the avocado crop.

Recommendations for future research

- Examine the suitability of different species of native vegetation that grow in the environ of avocado orchards for *A. l. lutescens* nymph survival and development to adults
- Examine movement patterns of adults between horticultural crops and between native vegetation to avocado crops. Examine distances that individual adults move within determined time frames.
- Establish economic thresholds for avocado crops based on pheromone trap catches so that insecticides can be applied prudently.

Refereed Scientific Publications

Conference abstracts:

Lindsay, K. R., Zalucki, M. P., Newton, I. R., Furlong. M.J. An Analysis of Banana-Spotting Bug Activity in Avocado Crops from Fruit-Set to Harvest. International Chemical Ecology Conference. 19-23 August 2013, Melbourne, Australia

Lindsay, K. R., Zalucki, M. P., Newton, I. R., Furlong. M.J. Effect of host plants on development, survival and fecundity for *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae). International Horticultural Congress. 17-22 August 2014, Brisbane, Australia

Lindsay, K. R., Zalucki, M. P., Newton, I. R., Furlong. M.J. 'Spatial and temporal dynamics of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) in avocado crops' Australian Entomological Society Conference. 28 September – 1 October 2015. Cairns, Australia

Lindsay, K. R., Zalucki, M. P., Newton, I. R., Furlong. M.J. 'Spatial and temporal dynamics of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) in avocado crops' Entomological Society of America Conference. 15-18 November 2015, Minneapolis, United States of America.

Submitted for publication:

Lindsay, K. R and Furlong, M. J. Development, survival and fecundity of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) on distinct phenological stages of different crop host plants. Journal of Economic Entomology. (Submitted, March 2016)

Lindsay, K. R. The ecology and behavior of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) on avocado crops. Ph. D thesis. University of Queensland. Brisbane (Submitted May 2016).

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Appendices

Appendix 1. Development, survival and fecundity of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) on distinct phenological stages of different crop host plants.

Appendix 2. Sampling efficiency and bias of pheromone traps capturing *Amblypelta lutescens lutescens* (Hemiptera: Coreidae).

Appendix 3. Population monitoring and spatial distributions of *Amblypelta lutescens lutescens* Distant (Hemiptera: Coreidae) in avocado crops.

Appendix 4. Potential applications of pheromone traps for IPM management of *Amblypelta lutescens lutescens* (Hemiptera: Coreidae) in avocado crops.

Appendix 1. Development, survival and fecundity of *Amblypelta lutescens lutescens* (Hemiptera: Coreidae) on distinct phenological stages of different crop host plants.

Methods

Laboratory A. l. lutescens culture

A laboratory culture of *A. l. lutescens* was established in the summer (November – May) before the study by collecting wild *A. l. lutescens* from *Murraya paniculata* hedges around Mareeba, Queensland (-16.9921ºS, 145.4067ºE). The culture was maintained in a glass aquarium (100 cm x 30 cm x 30 cm) in a controlled environment room (27°C (±0.5°C), 70% RH (±10%), 14: 10 L: D photoperiod) and supplemented annually with field-collected insects. Insects were fed on commercially sourced green bean pods and papaya (c.v Hybrid 1B) seedlings that were grown in a glasshouse in 10 cm diameter pots containing 50:50 peat moss and course sand potting mix.

Host plants and timing of experiments

In Far North Queensland, avocado crops (c.v Shepard and Hass) flower and set fruit during spring (August-October), undergo periods of vegetative flush in spring (September–October) and summer (January–April) and produce mature fruit in late summer (February– March) (Newett and Dixon 2010). Although lime crops in the region produce flowers, vegetative flush and fruit at multiple times during the year, the main lime fruit harvest is from January – March (Sunraysia Citrus Growers 2007), at the same time as avocado crops are harvested. In contrast to avocado and lime, papaya plants can produce flowers, vegetative flush and fruit simultaneously at all times of the year (Jimenez et al. 2013).

Experiments were designed based on the phenology of avocado crops; they started in September and ran through to February. Plant parts for experiments were cut from three-year-old avocado (c.v Shepard) trees and three-year-old lime (c.v Tahitian) trees at Walkamin Research Station, Queensland, Australia (-17.1380°, 145.4281°) and from two-year-old papaya trees (c.v Hybrid 1B) on a commercial papaya orchard located 15 km NWW of Mareeba, Queensland, Australia (-16.9837°, 145.3289°). Experiments on the suitability of vegetative flush and flowers were conducted in September. Single avocado panicle inflorescences and lime and papaya cymose inflorescences were removed from the plants using secateurs. All inflorescences were cut approximately 10 cm from the tip and only closed flowers were removed from the plants for experiments. Avocado, lime and papaya vegetative flush shoots were removed 10 cm from the shoot tips. Avocado and lime shoots were removed from branches located all over the trees tree while papaya vegetative flush shoots were only removed near the apex of the trees as new shoots were only situated near the apex. Experiments on the suitability of different sized fruit tested the suitability of small avocado (1-3 cm diameter at the widest point), small lime (1-2 cm) and small papaya (2-4 cm) fruit in October. Medium avocado (6-8 cm), medium lime (3-4 cm) and medium papaya (8-12 cm) fruit were investigated in November and large avocado ($\geq$12 cm), large lime ($\geq$5 cm) and large papaya ($\geq$15 cm) were investigated in February. Experiments to test the suitability of green bean pods were conducted at the same time as vegetative flush experiments in September.

Survivorship and development of A. l. lutescens on different phenological stages of different host plants

All experiments on avocado, lime, papaya and green beans were conducted in a controlled

environment room (27°C (±0.5°C), 70% RH (±10%), 14: 10 L: D photoperiod). Prior to each experiment, 20-30 pairs of adult male and female *A. l. lutescens* were isolated from the culture. Individual pairs were kept in 250 ml containers (Sarstedt) with a single 3 cm x 3 cm segment of the lid removed and replaced with metal gorse melted to the lid for ventilation. Individual pairs of insects were provided with green beans as a food source. Eggs were collected daily from each container and placed into a ventilated 250 ml container (Sarstedt). In experiments investigating the suitability of flowers, vegetative flush and fruit of different sizes, the relevant plant parts were placed in ventilated 250 ml containers (Sarstedt), large fruit were placed in 500 ml containers (Katermaster) with single 5 cm x 5 cm segments of the lid removed and replaced with metal gorse melted to the lid for ventilation. Single newly hatched neonate nymphs (< 24 h old) were distributed equally among the treatments daily and, depending on the availability of nymphs, approximately 30 neonates were reared separately on each plant part. Three replicates (n≈30 neonates per replicate) were prepared for each plant part treatment. Flowers and vegetative flush material were replaced every second day while fruit were replaced every five days. Insects were checked daily and mortality and stage of development recorded.

Adult weight and egg production

When insects completed the final molt they were individually weighed (< 24 h after molt) using a four decimal point scale in milligrams (HR-250AZ, A&D Limited) and their weights recorded. Male and female insects that had completed pre-imaginal development on the same food source were then paired in ventilated 250ml containers (Sarstedt), supplied with the same plant on which they had developed and incubated in the same controlled environment room used for nymph survival and development experiments. The plant parts and containers were then checked daily and any eggs that were laid were recorded; each pair of insects was monitored for a further 21 days following the first oviposition event.

Data analysis

Data were analysed using GraphPad Prism version 6 for Mac OS X (GraphPad Software, San Diego, California, USA; www.graphpad.com). Bartlett's test was used to determine if data were normally distributed. Data on the proportion of nymphs that survived from first instar to imago and proportion of nymphs that survived each instar were not normally distributed. Therefore, arcsine transformations were applied to the proportion data and transformed data were subject to one-way analysis of variance (ANOVA) tests, and significant differences between treatments were determined using a Tukey's range test. Data on the developmental time from first instar to imago and developmental time at each instar were not normally distributed. These data were subject to a non-parametric Kruskal-Wallace test when there were three or more treatments. A Mann-Whitney test was used to analyse developmental time of nymphs when there were only two treatments to compare. The weight of post imago adults (<24 hours) between sexes and host plant treatments were analysed using a 2-way ANOVA test with significant differences between treatments determined using a Tukey's range test. The pre-oviposition period of females (time in days from adult ecolosion to producing their first egg) and total number of eggs per female between treatments was analysed using a Kruskal-Wallace ANOVA test with significant differences determined by a Tukey's range test.

Results

Survivorship and development of *A. l. lutescens* on different phenological stages of different host plants

Flowers

High proportions (83%) of neonate *A. l. lutescens* were able to complete development to imago while feeding on papaya flowers (Table 1.1). No neonate nymphs completed development to imago while feeding on avocado or lime flowers (Table 1.1). High proportions of nymphs survived the first instar on lime (92%) and avocado flowers (82%). However, very low survival was recorded during the second instars on lime (7%) and avocado (3%) and no nymphs survived past the third instar on avocado flowers and fourth instar on lime flowers (Table 1.1). There was no significant difference in the proportion of nymphs surviving the first instar ($F_{2,6}=1.795$, $p=0.245$). The proportion of nymphs surviving the second instar was significantly higher on papaya flowers compared to avocado and lime flowers ($F_{2,5}=121.3$ $p<0.001$). There was no significant difference in the developmental period of first instar nymphs between treatments ($F_{3,207}=5.17$ $p=0.075$) (Table 2). The developmental period for the second instar was significantly shorter on papaya flowers than avocado and lime flowers ($F=23$, $p<0.001$) (Table 1.2).

Vegetative flush

Papaya flush and green beans were most suitable for *A. l. lutescens* development to imago; approximately 40-50 % of test insects developed from neonate to imago on these food sources (Table 1.1). High proportions of nymphs survived the first instar on lime flush (95%) but few (4%) completed development to the imago. No neonate nymphs survived to imago on avocado flush (Table 1.1). A high proportion of nymphs survived the first instar (92%) but no nymphs survived the second instar (Table 1.1). Overall, significantly higher proportions of nymphs survived from first instar to imago on green beans and papaya flush compared to lime flush ($F_{2,6}=13.95$, $p=0.006$) (Table 1.1). There was no significant difference in the proportion of nymphs that survived the first instar between treatments ($F_{3,8}=3.07$, $p=0.092$) (Table 1.1). There were significant differences in the proportions of nymphs that survived the second instar ($F_{3,8}=67.05$ $p<0.001$) with the highest proportions of nymphs surviving on green beans. There was no significant difference in the proportion of nymphs that survived the third instar between treatments ($F_{2,6}=1.48$, $p=0.300$) (Table 1.1). Significantly higher proportions of nymphs survived the fourth instar on papaya flush and green beans compared to lime flush treatments ($F_{2,6}=60.52$, $p=0.001$) (Table 1). Significantly higher proportions of nymphs survived the fifth instar on green beans compared to papaya flush and lime flush treatments ($F_{2,6}=6.52$, $p=0.031$) (Table 1.1).

The overall developmental period from first instar to imago was significantly shorter on papaya flush and green beans compared to lime flush ($F_{3,69} = 8.55$ $p=0.014$) (Table 1.2). There is some evidence to suggest significant differences in the developmental period of first instar nymphs between treatments ($F_{4,276} = 8.07$, $p=0.050$) (Table 1.2). Developmental period was significantly shorter on papaya flush and green beans compared to lime flush for the second instar ($F_{3,121}$ $F=7.90$, $p=0.019$), third instar ($F_{3,98} = 9.67$ $p=0.008$), fourth instar ($F_{3,80} = 9.19$ $p=0.011$) and fifth instar ($F_{3,66}=6.18$ $p=0.050$) (Table 1.2).

Fruit

No *A. l. lutescens* nymphs survived to imago while feeding on fruit of any test host plant. Some nymphs feeding on fruit were able to molt to the second instar on avocado, lime and papaya fruit, but none developed further. Lower proportions survived the first instar on fruit compared to vegetative flush and flowers for avocado (11-24%) lime (58-73%) and papaya (4-46%). Significantly higher proportions of nymphs survived on small sized ($F_{2,6}=17.16$ $p=0.003$) and medium sized ($F_{2,6}=8.93$

p=0.016) papaya and lime fruit compared to avocado fruit (Table 1.1). Significantly higher proportions of nymphs survived on large sized avocado fruit and lime fruit compared to papaya fruit ($F_{2,6}=14.42$, $p=0.005$) (Table 1). There was no significant difference in first instar developmental period for small sized fruit ($F_{3,78}=4.462$, $p=0.107$). The first instar developmental period was significantly different between treatments ($F_{3,81}=8.73$ $p=0.012$) with lowest developmental period observed on lime treatments. There was no significant difference in first instar developmental period between large sized fruit treatments ($F_{3,53}=0.23$, $p=0.893$) (Table 1.2). After each experiment on different size fruit, five fruit were randomly selected from each treatment and cut into thin cross sections (<3mm). There was observable evidence of feeding damage on avocado fruit.

Adult weight and egg production

Newly emerged adult females were significantly heavier than newly emerged adult males ($F_{1,120}=55.14$ $p<0.001$) (Table 1.3). Adults feeding on green beans were significantly heavier than adults feeding on papaya flowers and flush ($F_{2,120}=20.08$, $p=<0.001$) (Table 1.3). The mean pre-oviposition period was shorter when females fed on papaya flowers and green beans than when they fed on papaya flowers ($F_{3,60}=37.96$ $p=0001$). Females feeding on green beans and papaya flush also laid significantly more eggs thanks females feeding on papaya flowers ($F_{2,52}=44.7$, $p>0.001$) (Table 1.3).

Table 1.1. Mean percentage (±SE) of nymphs surviving at each instar stage when fed avocado, lime or papaya plant structures (3 replicates, n= 25-35 nymphs per replicate).

Host Plant	Plant structure	Percentage of original cohort surviving (±SE)					
		1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	Survival to imago
Avocado	Flowers	81.8 (±6.8)a	2.6 (±2.6)a	0	-	-	-
Lime		92.1 (±2.5)a	6.9 (±1.1)a	33.3 (±33.3)	-	-	-
Papaya		93.9 (±3.1)a	93.3 (±1.3)b	95.7 (±2.5)	96.8 (±3.2)	93.9 (±1.7)	82.9 (±4.9)
Avocado	Flush	91.7 (± 3.4)a	0	-	-	-	-
Lime		94.6 (± 3.7)a	38.6 (±8.6)a	65.6 (±8.7)a	43.3 (±3.3)a	33.3 (±16.7)a	3.8 (±2.1)a
Papaya		90.6 (± 3.4)a	71.2 (±3.8)b	71.9 (±21.7)a	89.4 (±5.4)b	73.3 (±12.5)a	41.6 (±11.7)b
Green Bean	Pod	70.8 (±11.3)a	80.7 (±5.4)b	92.5 (±5.3)a	98.3 (±1.7)b	96.7 (±3.3)b	47.1 (±3.8)b
Avocado	Small fruit	11.6 (±2.8)a	0	-	-	-	-
Lime		58.0 (±8.0)b	0	-	-	-	-
Papaya		28.9 (±7.7)b	0	-	-	-	-
Avocado	Medium fruit	23.5 (±7.2)a	0	-	-	-	-
Lime		73.0 (±9.2)a	0	-	-	-	-
Papaya		45.5 (±14.3)a	0	-	-	-	-
Avocado	Large fruit	19.1 (±2.4)a	0	-	-	-	-
Lime		56.1 (±5.6)a	0	-	-	-	-
Papaya		4.2 (±4.2)b	0	-	-	-	-

Different letters within columns for each plant structure represent significant differences between treatments ($p<0.05$). Third instar survival of nymphs was not statistically analysed between lime flowers and papaya flowers due to the lack of survival data collected on lime flowers.

Table 1.2. Median and range of developmental period (days) of nymphs from first instar to imago and developmental period of nymphs for each instar when fed avocado, lime and papaya plant structures.

Host plant	Plant structure	Developmental period (days) of each cohort range)					
		1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	Total
Avocado	Flowers	3 (2-4)a n=62	15 n=1	-	-	-	
Lime		3 (2-5)a n=71	20 (10-22)a n=5	9 n=1	-	-	
Papaya		3 (2-4)a n=74	8 (5-24)a n=70	8 (3-12) n=66	10 (5-21) n=64	12 (4-26) n=62	n=62
Avocado	Flush	3 (3-4)a n=80	-	-	-	-	-
Lime		3 (2-5)a n=75	13 (7-29)a n=29	12 (5-26)a n=17b	17 (11-21)a n=5	15.5 (11-20)a n=3	59 (53-65)a n=3
Papaya		3 (2-5)a n=61	11.5 (5-25)ab n=44b	7 (3-38)a n=37b	6 (4-37)b n=33	9 (7-19)a n=23	33.5 (27-80)b n=23
Green bean	Pods	3 (3-6)a n=59	9 (4-26)b n=48	12 (5-26)a n=44	8 (4-18)b n=42	9 (7-14)a n=41	41 (24-77)ab n=41
Avocado	Small fruit	4 (4-4)a n=10	-	-	-	-	-
Lime		4 (3-6)a n=45	-	-	-	-	-
Papaya		4 (3-5)a n=21	-	-	-	-	-
Avocado	Medium fruit	4 (3-5)a n=15	-	-	-	-	-
Lime		3 (2-4)b n=49	-	-	-	-	-
Papaya		4 (2-4)ab n=18	-	-	-	-	-

Avocado	Large fruit	3 (3-4)a n=14	-	-	-	-	-
Lime		3 (2-4)a n=3	-	-	-	-	-
Papaya		3 (2-4)a n=3	-	-	-	-	-

Different letters within columns for each plant structure represent significant differences between treatments ($p<0.05$). Second instar developmental time of nymphs on avocado flower treatments and third instar developmental time on limes were not compared statistically as there was no replication. Significant differences using Kruskal-Wallace test were detected in 1st instar developmental period on vegetative flush treatments. However, multiple comparisons tests did not detect significant differences between treatments.

Table 1.3. Mean weight (mg) (± SE) and of newly emerged (<24 hours) adult *A. l. lutescens* on different host plant sources.

Plant structure	Adult weight (mg)	Pre-oviposition period (days)	Total eggs (over 21 days)
Lime flush	368.3 (± 8.1) n=3	-	-
Papaya flowers	450.5 (±9.8)a n=63	31.9 (±0.2)a n=28	4.6 (±0.5a n=28
Papaya flush	461.5 (±18.3)a n=26	13.2 (±0.5)b n=11	38.6 (±6.05)b n=11
Green beans	550.6 (±15.5)b n=40	14.7 (±1.2)b n=17	47.8 (±5.9)b n=17

Different letters between columns represent significant differences between treatments ($P<0.05$) There was no interaction significant interaction between adult sex and host plant treatments ($F_{2,120}=0.16$, $p=0.984$). Lime weight was not included in the statistical analysis.

Appendix 2. Sampling efficiency and bias of pheromone traps capturing *Amblypelta lutescens lutescens* (Hemiptera: Coreidae).

Methods

Laboratory colony

A previously established laboratory colony of *A. l. lutescens* was used to source adult and nymphs for cage experiments. The colony had been established by collecting wild *A. l. lutescens* from *Murraya paniculata* hedges around Mareeba, Queensland (-16.9921°, 145.4067°). The colony was maintained in a glass aquarium (100 cm x 30 cm x 30 cm) and fed papaya seedlings (yellow) and commercially sourced green bean fruit. Papaya seedlings were grown and maintained in a glasshouse in 10 cm diameter pots with a 50:50 mixture of peat and coarse gravel and watered twice daily. The colony was maintained in a controlled temperature room (27°C (±0.5 °C), 70% humidity (±10%), and a 14:10 L:D) at the Department of Agriculture and Fisheries (DAF) facility at Mareeba.

Proportions of nymphs: adults and males: females caught in pheromone traps and estimated by insecticide fogging

Field site

The field trial was conducted on a mango block (0.6 Ha) made up of 20 different hybrid varieties crossed with cv Kensington Pride used for a mango breeding program at Southedge Research Station on the Atherton Tablelands (-16.9781°, 145.3451°). The mango trees were seven years old. There were eight rows of trees and 36-46 trees per row. The mango trees were spaced 3 m apart and the canopies of neighbouring trees overlapped There were some large gaps throughout the mango block as a result of mango trees dying and being cleared away.

Insecticide application

Insecticide knock-down sampling sessions took place on five occasions on 10/11/2014, 24/11/2014, 12/01/2015, 23/02/2015 and 17/03/2015. These dates were selected based on high numbers of *A. l. lutescens* captured in pheromone traps near those dates to maximize insecticide knock-down sampling efficiency. The dates were also selected on days with low wind forecasts. The synthetic pyrethroid *β*-cyfluthrin (Bulldock®, Bayer®) is registered for the control of *A. l. lutescens* on mango orchards (APVMA 2015) and was used to sample *A. l. lutescens* in the mango trees. The concentration ratio of *β*-cyfluthrin to water followed instructions for *A. l. lutescens* control of 50ml to 100 litres or at a ratio of 0.0005:1 during the study. During the first two tarpaulin sampling sessions, *β*-cyflurthrin was applied to all mango trees in the block using an air-blast sprayer behind a tractor as part of a pest management program to reduce *A. l. lutescens* damage on mango fruit. However pest management was ceased during the current study. To continue with insecticide knock-down sampling, *β*-cyflurthrin was applied using a Solo® 10 litre backpack sprayer to mango trees used for sampling only. The different pesticide application methods during the study were accounted for during statistical analysis. During each insecticide knock down sampling session, a small number of mango trees with pheromone traps (trap trees) were selected to be sampled and an equal number of mango trees without pheromone traps (non-trap trees) were selected to be sampled (Figure 2.1). The number of trap trees and non-trap trees sampled with insecticide knock down varied on some sampling dates. On the first insecticide knock-down sampling session, three trap trees and three non-trap trees were sampled (T1-3, N1-3) (Figure 2.1). On the second occasion, four trap tree and four non-trap trees were sampled (T1-4, N1-4) and on the last three occasions, five trap trees and five non-trap trees were sampled (T1-5, N1-5) (Figure 2.1). The increase in the number of trees sampled over time was due to improved labour efficiency.

Figure 2.1. Field site used for study (T=mango trees with pheromone traps and N= mango trees without pheromone traps sampled using insecticide knock down.

Pheromone trap sampling

A prototype trap has been designed by the Department of Agriculture and Fisheries in Queensland that uses the major chemical components emitted by male *A. l. lutescens* as an attractant lure. The trap consists of green twin-walled polypropylene sheeting (Corflute®) covered by double sided tape coated with acrylic adhesive. Thin wire is used to secure the rubber septa with the pheromone in a hole at the centre of the trap. Twelve pheromone traps were distributed in selected rows across the mango block with spacing distances of approximately 25-60m between traps. Pheromone trap catch data were collected weekly for 22 weeks from 30/10/2014 – 26/03/2015 by recording adult males, females, and nymphs of each instar captured in the pheromone traps. Pheromone traps were changed once a fortnight due to the surface adhesive degradation occurring under UV light. Earlier field trials found the maximum attractiveness of the artificial pheromone lure lasted six weeks in the field (HAC Fay unpubl. 2011); therefore the pheromone septa in each pheromone trap were changed every six weeks to minimize the loss of attractiveness.

Insecticide knock down sampling

Prior to insecticides being applied to the mango block, blue tarpaulins (6m x 3m) were positioned under trap tree and non-trap tree mango trees by cutting the tarpaulins down the middle to position the tree trunk in the middle of the tarpaulin. Tent pegs were used to secure the tarpaulins underneath the mango trees. The edge of the tarpaulins extended to the tree trunks of neighbouring trees, therefore each tarpaulin sampled the half the canopy of the two neighbouring trees. β-cyfluthrin was applied to trap trees and non-traps to kill all *A. l. lutescens* when wind conditions were calm and settled at approximately 0700. Approximately one hour after insecticide applications, trap trees and non-trap trees were shaken vigorously for one minute and tarpaulins were inspected for ten minutes. All adult males, adult females, and nymphs of each instar that dropped onto the tarpaulins were recorded.

Pheromone trap data was collected weekly for 22 weeks while insecticide knock-down data was collected on five occasions during the study. Adult male/female and nymph/adult proportions were analysed from each pheromone trap and tarpaulin. To account for different sampling regimes between the sampling methods for analysis, data collected on each pheromone trap was cumulated in time periods between each insecticide knock-down sampling session.

Proportions of nymphs: adults captured in pheromone traps in cage experimental conditions

Two insect cages (3m x 2m x 2m) were set up inside a glasshouse at the Department of Agriculture and Fisheries (DAF) research facility in Mareeba, Queensland. One cage was used as a treatment cage and had a pheromone trap with the attractant lure while the other cage was used as a control cage and had a trap without the attractant lure. In each cage, two avocado seedlings were placed in the middle of the cage with a pheromone trap wire wrapped around string attached to the cage frame between the avocado seedlings (Figure 2.2).

The cage experiment was only replicated three times due to insufficient *A. l. lutescens* colony populations. For each experimental replicate, 16 adult males, 16 adult females and 16 nymphs of each nymph instar were released into the cages approximately 24 hours before the experiment. The experiments began at 0600 with the placement of the pheromone traps and control traps in the cages and ended at 1800 on the same day. The traps in both treatment and control cages were checked every hour and any adult or nymph *A. l. lutescens* captured were recorded and removed from the traps. Cages used for treatments and controls were swapped per replicate.

Figure 2.2. Cage experiment set up with avocado seedlings and pheromone trap.

Data analysis

Data were analysed using GenStat 16 statistical package, version 16.1 (Genstat 16 committee 2013). For field studies, the proportion of males relative to females and proportion of nymphs relative to adults from pheromone trap data and insecticide knock-down sample replicates were compared using Generalised Linear Mixed Models (GLMM) with a binomial distribution and logit link function. Pheromone trap and insecticide knock-down samples were fitted as fixed terms. Potential natural variation in adult male/ female proportions or nymph/adult proportions over time and the potential influence of changes to insecticide knock-down

method during the study were accounted for by fitting the sample data and time as a random term in the GLMM. The mean number of adult males, females and nymphs of each instar stage sampled using insecticide knock-down on trap trees and non-trap trees were compared using a GLM with a Poisson distribution and logarithm link function. Over-dispersion (greater variability occurring than predicted) and under-dispersion (less variability in data than predicted) were accounted for by estimating the dispersion parameters. For cage experiments, the proportion of total *A. l. lutescens* captured in traps in treatment cages and control cages was compared using a GLM model with a binomial distribution and logit link function. The proportions of adult and nymph *A. l. lutescens* captured in traps in treatment cages and control cages was analysed separately using GLM models with a binomial distribution and logit link function. Dispersion in each GLM model was estimated per analysis.

Results

Proportions of males: females and nymphs: adults caught in pheromone traps and estimated by insecticide fogging

Adult male and female proportions

There were no significant differences in the proportion of adult males relative to adult females collected on tarpaulins under trap tree (0.46 ± 0.08, n=17) and non-trap tree (0.64 ± 0.12, n=12) insecticide knock-down treatments ($F_{1,20}=2.14$, $p=0.159$). Therefore, all insecticide knock-down samples were combined and compared with pheromone trap samples. There was a significantly lower proportion of adult males relative to females found on pheromone trap samples compared to proportions samples from insecticide knock-down ($F_{1,76}=5.69$ $p=0.020$) (Table 2.1). Using both pheromone trap data and insecticide knock down data, there was no significant difference in overall proportions of males relative to females over time ($F_{4,76}=1.42$, $p=0.235$) and no significant interaction between treatments over time ($F_{4,76}=0.45$, $p=0.776$). Therefore, time and changes in insecticide application method during the study did not have a significant impact on results.

Immature nymphs and adult proportions

There were no significant differences in the proportion of nymphs relative to adults collected on tarpaulins under trap tree (0.64 ± 0.04, n=19) and non-trap tree (0.68 ± 0.07, n=12) insecticide knock-down treatments ($F_{2,21.4}=1.39$, $p=0.270$). Therefore, all insecticide knock-down samples were combined and compared with pheromone trap samples. There were a significantly higher proportion of nymphs relative to adults from insecticide knock-down samples compared to pheromone trap samples ($F_{1,46.6}=59.57$ $p=<0.001$) (Table 2.1). Using all the data, nymph proportions relative to adults varied over time ($F_{4,40.9}=4.25$, $p=0.006$). Unprotected LSD tests indicate higher nymph proportions relative to adults on the 12/01/2015. There was no significant interaction between treatments over time ($F_{4,39.3}=0.54$, $p=0.706$). Therefore, time and changes in insecticide application method during the study did not have a significant impact on results.

Population structure sampled using insecticide knock-down

There were significantly more adult females sampled from mango trees with pheromone traps compared to mango trees without pheromone traps ($F_{1,8}=9.65$, $p=0.015$) (Figure 2.3). There was no significant difference in the number of adult males ($F_{1,8}=1.75$, $p=0.193$), first instar nymphs ($F_{1,8}=1.33$, $p=0.282$), second instar nymphs ($F_{1,8}=0.001$, $p=0.970$), third instar nymphs ($F_{1,8}=0.27$, $p=0.617$), fourth instar nymphs ($F_{1,8}=1.09$, $p=0.327$) and fifth instar nymphs ($F_{1,8}=0.27$, $p=0.620$) (Figure 2.3). Overall there were significantly more 2nd instar nymphs sampled compared to all other instars ($F_{4,20}=6.14$, $p=0.002$) (Figure 2.3).

Table 2.1. Mean (± SE) proportions of adult males relative to females sampled using pheromone traps and insecticide knock-down and proportions of immature nymphs relative to adults.

	Male/Female proportions	Nymph/adult proportions
Pheromone trap sampling	0.32 ± 0.03 n=57	0.29 ± 0.03 n=57
Insecticide knockdown sampling	0.54 ± 0.07 n=38	0.67 ± 0.05 n=30

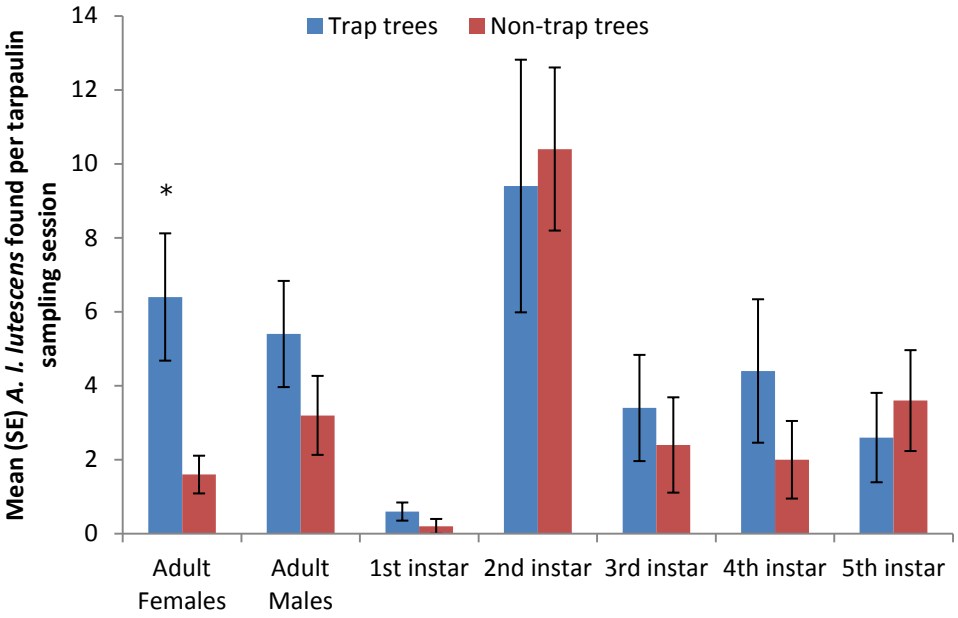

Figure 2.3. Mean (± SE) number of adults and different nymph instars sampled per on insecticide knock-down sessions (n=5).

Asterix (*) indicates statistically significant differences between trap tree and non-trap tree treatments ($p<0.05$)

Proportions of nymphs: adults captured in pheromone traps under cage experimental conditions

During the 12 h period, very small proportions of the adults and nymphs released were captured in traps with an attractant lure in treatment cages and in traps without an attractant lure in control cages (Table 2.2). There was no significant difference between the proportions of released *A. l. lutescens* captured in traps in treatment cages and control cages ($F_{1,4}=0.39$, $p=0.567$). There was no significant difference in the proportions of released adults captured in traps compared to nymphs in treatment cages ($F_{1,4}=1.31$, $p=0.316$) and control cages ($F_{1,4}=0.20$, $p=0.679$).

Table 2.2. Mean (± SE) proportions of *A. l. lutescens* adults and nymphs captured in treatment and control cages over 12 hour time period.

A. l. lutescens life stage	Proportion (± SE) caught in traps containing lure	Proportion (± SE) caught in traps without lure
Adults	0.06 ± 0.02	0.08 ± 0.02
Nymphs	0.07 ± 0.01	0.11 ± 0.06

Appendix 3. Population monitoring and spatial distributions of *Amblypelta lutescens lutescens* (Hemiptera: Coreidae) in avocado crops.

Methods

Field sites

Field trials were conducted on a commercial orchard situated 15km west of Walkamin, Australia (-17.1198°, 145.2880°). The region experiences mean temperatures and monthly rainfall of 30°C and 120-252mm during the summer months (December – February) and mean temperatures and monthly rainfall of 24°C and 13mm during the winter months (June – August) (Australian Goverment Bureau of Meteorology 2015). Avocado (c.v Shepard and Hass), limes (c.v Tahitian) and mangoes *Mangifera indica* L. (c.v Kensington Pride and R2E2) were grown on the same property. Every 7-14 days, registered chemical insecticides with active ingredients β-cyfuthrin and Methidathion were applied when avocado fruit were on the trees (October – March) using an airblast sprayer behind a tractor. This was done to reduce *A. l. lutescens* feeding damage.

Two avocado blocks (field A and field B) (c.v Shepard), approximately 100m apart, were selected for a two year study based on high *A. l. lutescens* feeding damage on avocado fruit observed by the grower in previous seasons. Field A was approximately 5.6 Ha and surrounded by dry sclerophyll forest and other avocados (c.v Hass) and limes (c.v Tahitian) (Figure 3.1). A seasonal creek flowed down the North Eastern boundary of field A during the wet season. Field B was approximately 4.2 Ha and was surrounded by dry sclerophyll forest and another avocado block (c.v Shepard) separated by a tarmac road. The Walsh River flowed down the North East boundary of Field B (Figure 3.1). One lime block selected for a one month study was approximately 2 Ha and surrounded by dry sclerophyll forest and an avocado crop (c.v Shepard) (Figure 3.1).

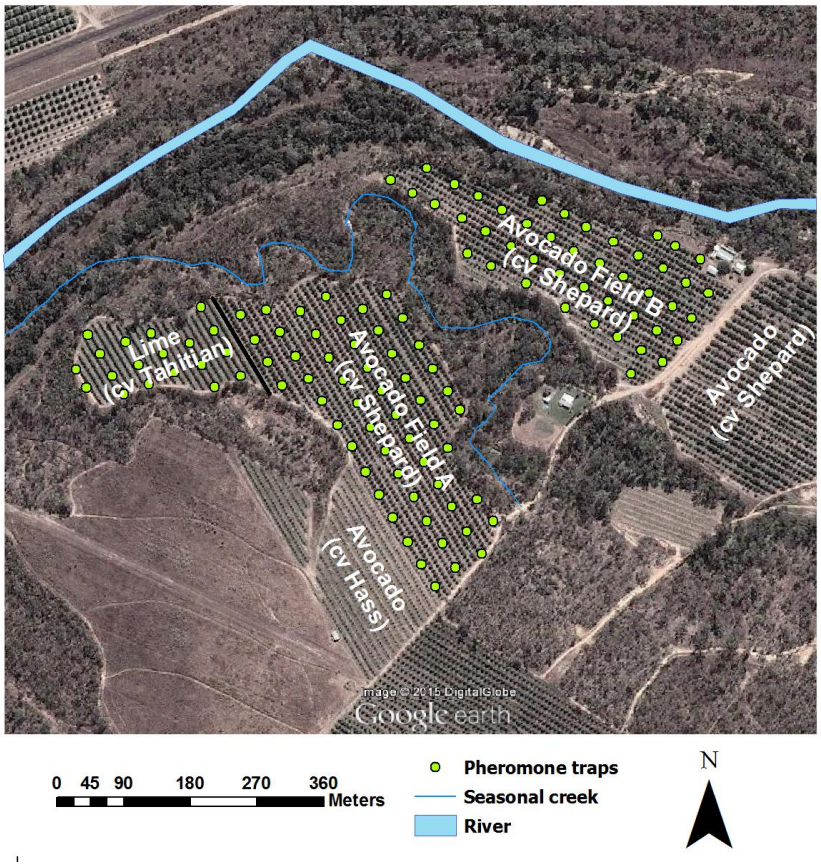

Figure 3.1. Avocado field A and B field sites and lime crop field site

Pheromone trap design

A prototype trap has been designed by the Department of Agriculture and Fisheries in Queensland that uses the major chemical components emitted by male *A. l. lutescens* as an attractant lure. The trap consists of green twin-walled polypropylene sheeting (Corflute®) covered by double sided tape coated with acrylic adhesive (Figure 3.1). The concentrations of each chemical component of the lure are currently under Intellectual Property protection. Thin wire is used to secure the rubber septa with the pheromone in a hole at the centre of the trap. Due to the pheromone trap adhesive degradation under UV exposure, the pheromone traps were changed once a fortnight to maintain trapping efficiency. Earlier field trials found the maximum attractiveness of the artificial pheromone lasted six weeks in the field (HAC Fay unpubl. 2011), therefore the pheromone septa in each pheromone trap were changed every six weeks.

Figure 3.2. Pheromone trap in an avocado orchard with captured adult *A. l. lutescens*.

Population monitoring

In the avocado crop, adult and nymph *A. l. lutescens* populations were sampled over a two year period from November 2013 on field A, December 2013 on field B until November 2015 on both field sites. Field A contained 56 pheromone traps and field B contained 45 pheromone traps (Figure 3.1). The lime crop contained 19 pheromone traps and *A. l. lutescens* populations were sampled over 4 week period during December 2013. Pheromone traps were arranged in a 36m by 36m grid on both avocado and lime crops (Figure 3.1). The pheromone trap grid was a continuation of the pheromone trap grid in field A. In both crops, the surface of the each pheromone trap was examined weekly and any adult males, adult females, and nymphs (instar recorded) captured in traps were recorded.

Data analysis

Population monitoring

In the avocado crop, the mean number of adults, second instar nymphs and third-fifth instar nymphs per pheromone trap was calculated per week. The mean number of adult males and females, mean number of nymphs and adults and mean number of second instar nymphs and third to fifth instar nymphs captured per week on each field site were statistically compared using Generalised Linear Models (GLM) with a Poisson distribution and logarithm link function (Genstat 16 committee 2013).

SADIE analysis

The position of each *A. l. lutescens* adult and nymph captured on the pheromone trap grids was recorded each week for spatial analysis. Spatial distributions were calculated using the Spatial Analysis by Distance Indices (SADIE) program. (SADIEShell, version 2.0,home.cogeco.ca/_sadiespatial/SADIEShell.html) (Perry 1995, Perry et al. 1999). The SADIE method uses count data and a relative sampling site coordinate system to calculate the spatial distribution based on the minimal distance that individuals in a population would need to expend to move to a completely regular arrangement in which abundance at each sampling unit was equal. At each sampling unit, a clustering index is assigned to determine whether the unit belonged to a population aggregation (>1.5) or spatial gap (<1.5) in their distribution. SADIE determines non-randomness on a field site by comparing observed spatial patterns with multiple random arrangements across the sampling area. Statistically significant population aggregations on a field site is determined by an associated probability calculated by SADIE based on the cluster indices under the null hypothesis of a random distribution when Pa < 0.025 or > 0.975 (uniformity).

In the avocado crop, SADIE spatial analyses were conducted on weekly pheromone trap data collected on adults, nymphs and total *A. l. lutescens* for a two-year period. SADIE spatial analyses were also conducted on year-end cumulated pheromone trap catch data for each year of the study on total *A. l. lutescens*, total adults, adult males, adult females and total nymphs. Spatial analyses were conducted on field A and field B separately. A SADIE spatial analysis requires count data from two or more locations to produce spatial statistics. If there were less than two geo-referenced sampling points with data, no analysis was undertaken. A SADIE spatial analysis was also conducted on adult, nymph and total *A. l. lutescens* captured in pheromone traps from the 01/12/2013-27/12/2013 on the lime block and from the avocado field A site for the same time period.

The SADIE association tool compares population aggregation indices for two sets of data calculated from the SADIE program and applies an index of association of either positive correlations (X>1) or negative correlations (X<0). Positive correlations indicate population aggregations overlap clusters and spatial gaps overlap gaps between the paired datasets. Negative correlations indicate that population aggregations and gaps overlap each other between the paired datasets (Perry and Dixon 2002). In the avocado crop, the spatial association was calculated between adults and nymphs for each year and field site, adult males and females for each year and field site, and adult, nymph and total counts by field site and between years. In the lime crop, the spatial association was calculated between adult and nymphs.

Spatial patterns and total density counts based on year-end cumulated pheromone trap catch data each year were visualised by importing total local cluster indices calculated by SADIE into ArcMap (version 10.0; Environmental Systems Research Institute [ESRI] 2014). Shape file masks were created for each field site and the Inverse Distant Weight (IDW) spatial statistical method was used to generate spatial interpolated maps. The IDW method calculates values in un-sampled locations based on weighted averages of known values within the neighbourhood. The IDW method assumes spatial autocorrelation in the data and is suitable for spatial distribution data (Lu and Wong 2008). The power setting which controls the significance of surrounding points on each interpolated value and the number of nearest points used to perform the interpolation were left on the default settings of two and 12.

Results

Population monitoring

On field A, a total of 1491 adults (1041 females and 450 males), 439 second instar nymphs, 12 third instar nymphs, 16 fourth instar nymphs and 11 fifth instar nymphs were captured during the study. Significantly more adult females were captured in pheromone traps per week than adult males ($F_{1,196}=32.22$, $p<0.001$) (Table 3.1). Significantly more adults were captured per week compared to nymphs ($F_{1,196}=54.20$, $p<0.001$) and significantly more second instar nymphs were captured per week compared to third-fifth instars combined ($F_{1,196}=110.18$, $p<0.001$) (Table 3.1). On field B, a total of 1517 adults (975 females and 542 males), 786 second instar nymphs, 12 third instar nymphs, 20 fourth instar nymphs and 9 fifth instar nymphs were captured during the study. Significantly more adult females were captured in pheromone traps per week than adult males ($F_{1,194}=20.18$, $p<0.001$) (Table 3.1). Significantly more adults were captured per week compared to nymphs ($F_{1,194}=14.35$, $p<0.001$) (Table 3.1). Significantly more second instar nymphs were captured per week compared to third-fifth instars combined ($F_{1,194}=45.52$, $p<0.001$) (Table 3.1). In the lime crop, there was significantly more adults were captured per week compared to nymphs ($F_{1,6}=6.84$, $p=0.04$) but no significant difference in the number of adult females caught in pheromone traps compared to adult males and number of second instar nymphs captured per week compared to third-fifth instars combined. ($F_{1,6}=0.31$, $p=0.596$) (Table 3.1).

During both years of the study on both avocado field sites, higher *A. l. lutescens* population densities were recorded during October – May and lower *A. l. lutescens* population densities were recorded during July-September (Table 3.1). In the 2013/2014 sampling year, peaks in the mean number of adults pheromone trap occurred on the 9th May on field A and 31st January on field B. In the 2014/2015 sampling year, peaks in the mean number of adults pheromone trap occurred on the 7th May on field A and 4th May on field B. Adult and nymphs densities recorded on both field sites were not influenced by avocado fruit phenology as high densities were recorded after fruit was harvested during both years of the study. Third, fourth and fifth instar nymphs were only captured in pheromone traps from March – May.

SADIE analysis – Avocado crop

On field A, adults were significantly aggregated on 12 out of 93 (13%) analyses, nymphs were significantly aggregated on 6 out of 53 (9%) analyses and total *A. l. lutescens* were significantly aggregated on 13 and of 93 (14%) analyses. On field B, adults were significantly aggregated on 5 out of 92 (5%) analyses, nymphs were significantly aggregated on 6 out of 54 (7%) analyses and total *A. l. lutescens* were significantly aggregated on 12 and of 92 (13%) analyse (Figure 3.5).

During both years of the study, SADIE analysis on cumulated year end data for adults identified significant aggregation occurring on field A (Table 3.1). High densities and population aggregations of adults were identified in an area on the North East boundary adjacent to the lime crop and native vegetation (Figures 3.6 and 3.8). A significant spatial gap was identified on the opposite side of the avocado block from the population aggregations in an area with low densities next to a block of Hass avocados (Figures 3.6 and 3.8). No significant population aggregations were identified for adults on field B during both years of the study. SADIE analysis on cumulated year-end data for nymphs identified significant population aggregations on field B during the first year of the study (Tables 3.1 and 3.2) High densities of nymphs and an aggregated cluster of nymphs were identified along the Northern boundary adjacent to native riparian vegetation (Figures 3.6 and 3.8). There was no significant aggregation of nymphs on field A during both years of the study. Separate SADIE analysis on adult males and females count data identified statistically significant aggregations in both male and female populations in field A but not in field B (Table 3.2).

The SADIE association tool found adult male and female spatial distributions were significantly spatially associated on both avocado field sites (Table 3.2). Adult and nymph spatial distributions were significantly associated on field B but not field A (Table 3.2). Spatial distributions of adults between years were

significantly associated on field A but not field B. Contrary to this, spatial distributions of nymphs were significantly associated on field B but not field A.

SADIE analysis – Lime crop

SADIE analysis on cumulated data for adults and total *A. l. lutescens* collected during December 2013 in avocado Orchard A and in the adjacent lime block identified significant aggregation (Table 3.1). Spatial aggregation was identified throughout the lime block and spatial gap were identified on field A (Figure 3.7). SADIE analysis for cumulated nymph data indicated no significant aggregation occurring (Table 3.2). The SADIE spatial association tool found no association between adults and nymph spatial distributions (Table 3.2).

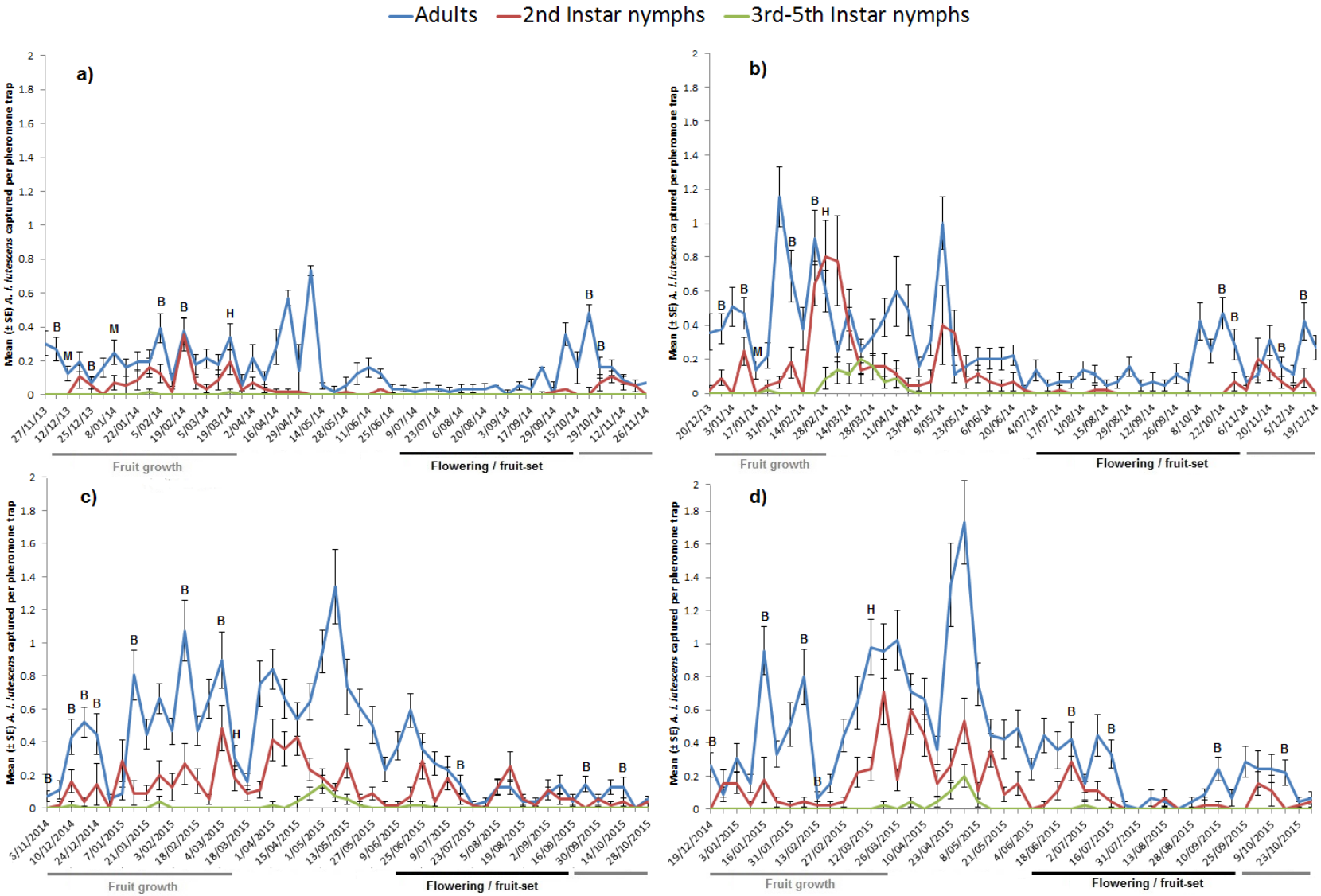

Figure 3.3. Mean (± SE) of *A. l. lutescens* adults and nymphs captured in pheromone traps in field A 2013- 2014 (a), field B 2013- 2014 (b), field A 2014-2015 (c), field B 2014-2015 (d). B=β-cyfluthrin application, M=Methidathion application, H=Crop harvested.

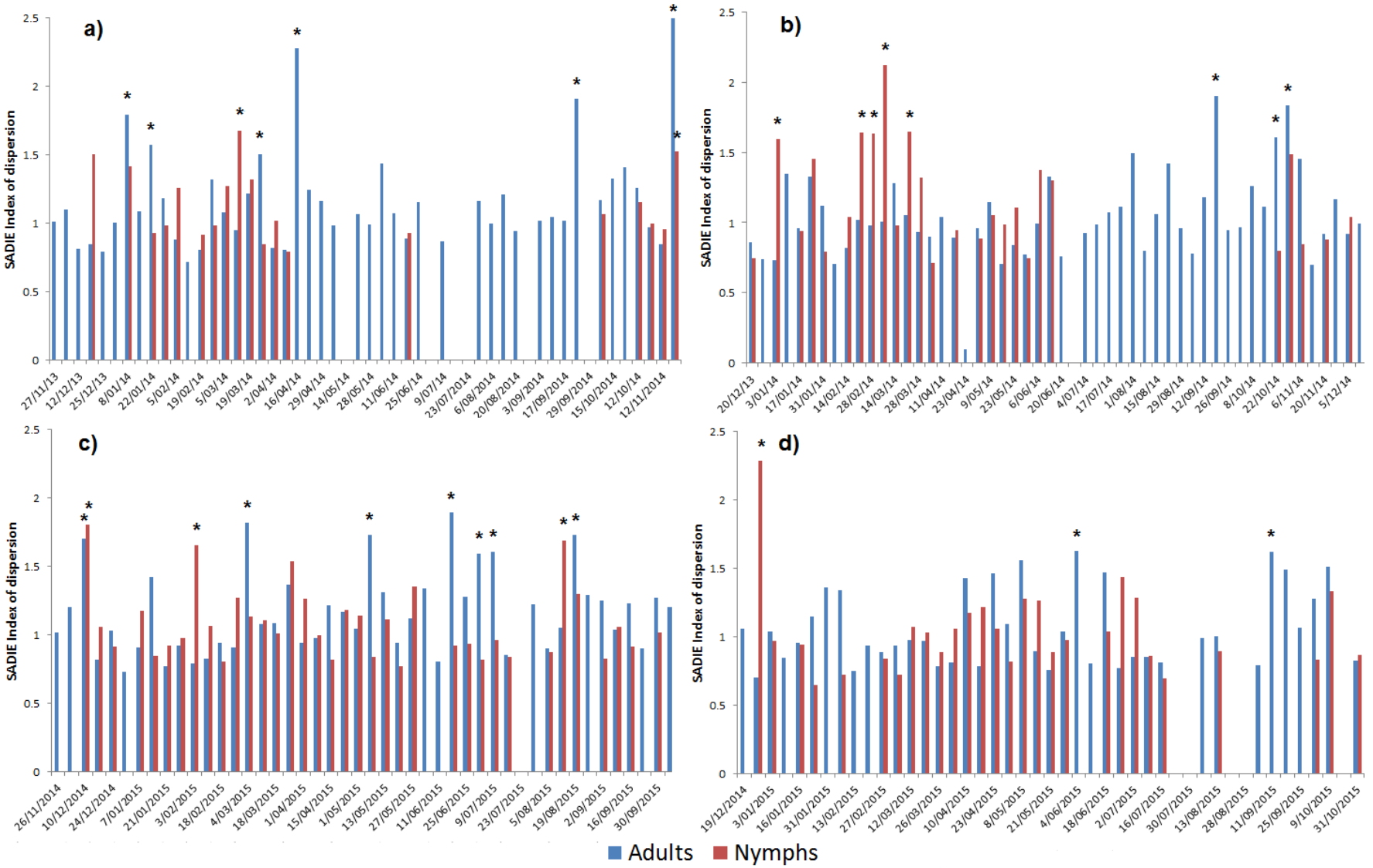

Figure 3.4. SADIE indices of dispersion over time for Orchard A 2013- 2014 (a), Orchard B 2013- 2014 (b), Orchard A 2014-2015 (c), Orchard B 2014-2015 (d). Significant spatial aggregations indicated by * ($p<0.025$).

Table 3.1. Mean (±SE) numbers of *A. l. lutescens* of different developmental stages captured in pheromone traps per week

	# Weeks	***Male adults***	***Female adults***	***Total adults***	***Total nymphs***	***2nd instar nymphs***	***3rd-5th instar nymphs***
Orchard A	100	4.5 ± 0.5	10.5 ± 1.1	15.1 ± 1.5	4.7 ± 0.6	4.6 ± 0.6	0.3 ± 0.1
Orchard B	99	5.3 ± 0.6	10.1 ± 0.9	15.5 ± 1.3	5.9 ± 0.9	5.3 ± 0.8	0.6 ± 0.2
Limes	5	4.2 ± 1.2	7.1 ± 2.4	11.3 ± 3.3	2.3 ± 1.3	1.5 ± 1.2	0.8 ± 0.8

Table 3.2. Year-end SADIE statistics from *A. l. lutescens* adult and nymph count data.

Field site	Time period	Total		Adult		Nymph		Adult/Nymph association	
		Ia	***Pa***	***Ia***	***Pa***	***Ia***	***Pa***	***X***	***P*(x)**
Field A	2013/2014	2.476	0.0002	2.528	0.0002	1.492	0.0458	0.2430	0.0449
Field A	2014/2015	1.775	0.0089	1.996	0.0020	1.027	0.3504	0.1939	0.1089
Field B	2013/2014	1.732	0.0146	1.296	0.1193	1.781	0.0107	0.3931	0.0081
Field B	2014/2015	1.064	0.3089	1.091	0.2745	1.007	0.3772	0.6869	<0.0001
Limes + Field A	Dec 2013	2.554	<0.0002	2.697	0.0002	1.079	0.3142	0.1793	0.0866

Ia = Overall index of dispersion indicating aggregated (>1), random (1) or uniform (<1) pattern Significance in aggregation determined by $a = 0.05$ ($P < 0.025$ or $P > 0.975$).

Pa = p-value for null hypothesis of spatial randomness.

X = Overall index of aggregation between each paired dataset. Significance in association is positive for $X > 0$ ($P < 0.025$) or negative for $X < 0$ ($P > 0.975$).

Table 3.3. Spatial associations of adult, nymph and total *A. l. lutescens* populations on each avocado field site between 2013/2014 and 2014/2015 datasets.

Field site	***Total***		***Adult***		***Nymph***	
	X	***P*(x)**	***X***	***P*(x)**	***X***	***P*(x)**
Orchard A	0.1932	0.1091	0.3195	0.0082	0.0192	0.4577
Orchard B	0.4035	0.0052	0.2243	0.0867	0.3243	0.01759

Table 3.4. Year-end SADIE statistics from adult male and female count data on each avocado field site

Field site	Year	Female		Male		Female/Male Association	
		Ia	Pa	Ia	Pa	X	P(x)
Field A	**2013/2014**	2.303	0.0002	2.153	0.0005	0.4398	0.0003
Field B	**2013/2014**	0.936	0.4937	1.511	0.0551	0.3823	0.0045
Field A	**2014/2015**	1.849	0.0045	1.912	0.0023	0.5423	0.0005
Field B	**2014/2015**	1.167	0.2009	0.900	0.5716	0.4049	0.0047

Ia = Overall index of dispersion indicating aggregated (>1), random (1) or uniform (<1) pattern Significance in aggregation determined by *a* = 0.05 (P < 0.025 or P > 0.975).

Pa = p-value for null hypothesis of spatial randomness.

X = Overall index of aggregation between each paired dataset. Significance in association is positive for X > 0 (P < 0.025) or negative for X < 0 (P > 0.975).

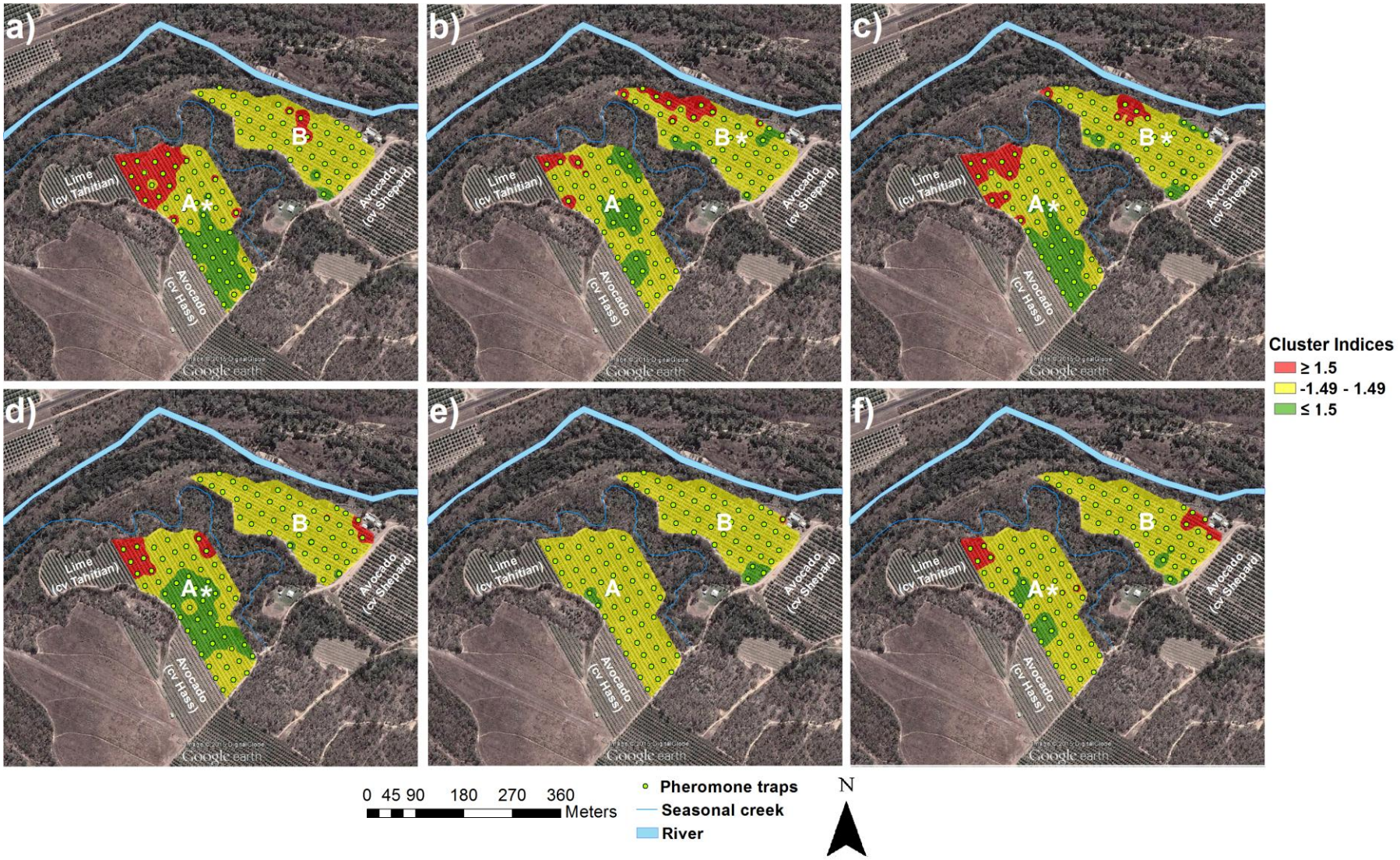

Figure 3.5. Spatial interpolations of SADIE local cluster indices on field A and field B for *A. l. lutescens* adults during the 2013/2014 season (a), *A. l. lutescens* nymphs during the 2013/2014 season (b), total *A. l. lutescens* during 2013/2014 season (c), adult *A. l. lutescens* during the 2014/2015 season (d), nymph *A. l. lutescens* during the 2014/2015 season (e) and total *A. l. lutescens* during the 2014/2015 season. Significant spatial aggregations on each field site indicted by *

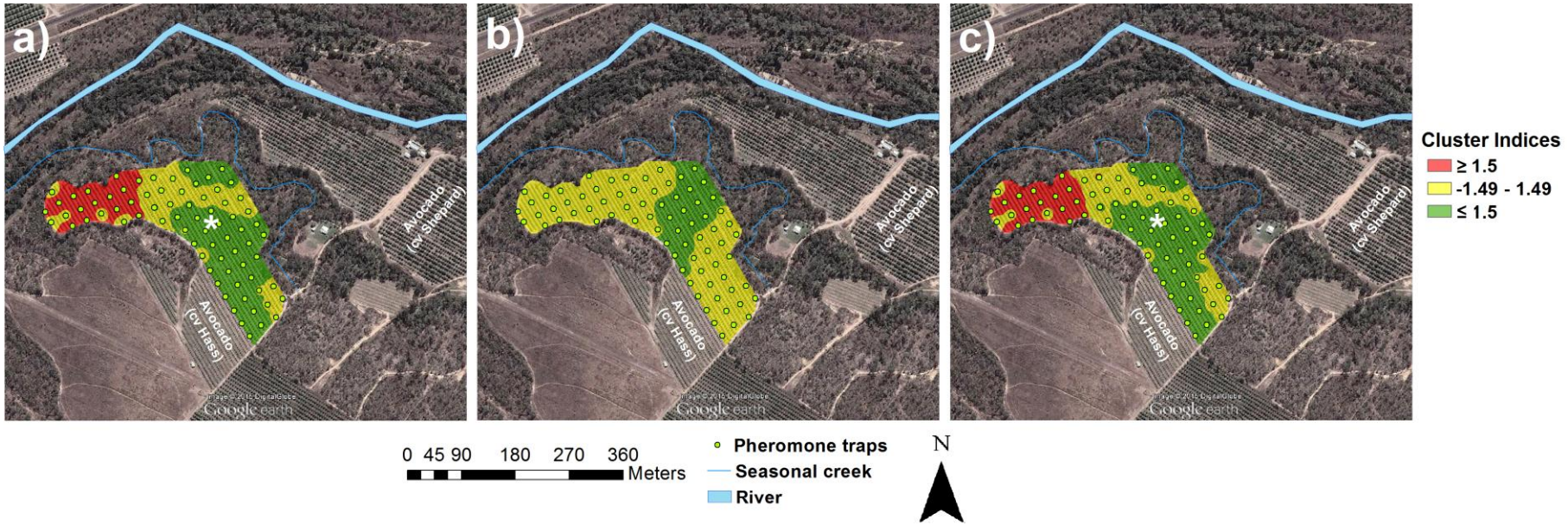

Figure 3.6. Spatial interpolations of SADIE local cluster indices on Orchard A and the lime block for *A. l. lutescens* adults during December 2013 (a), *A. l. lutescens* nymphs during December 2013 (b) and total *A. l. lutescens* during December 2013 (c). Significant spatial aggregations on each field site indicted by *

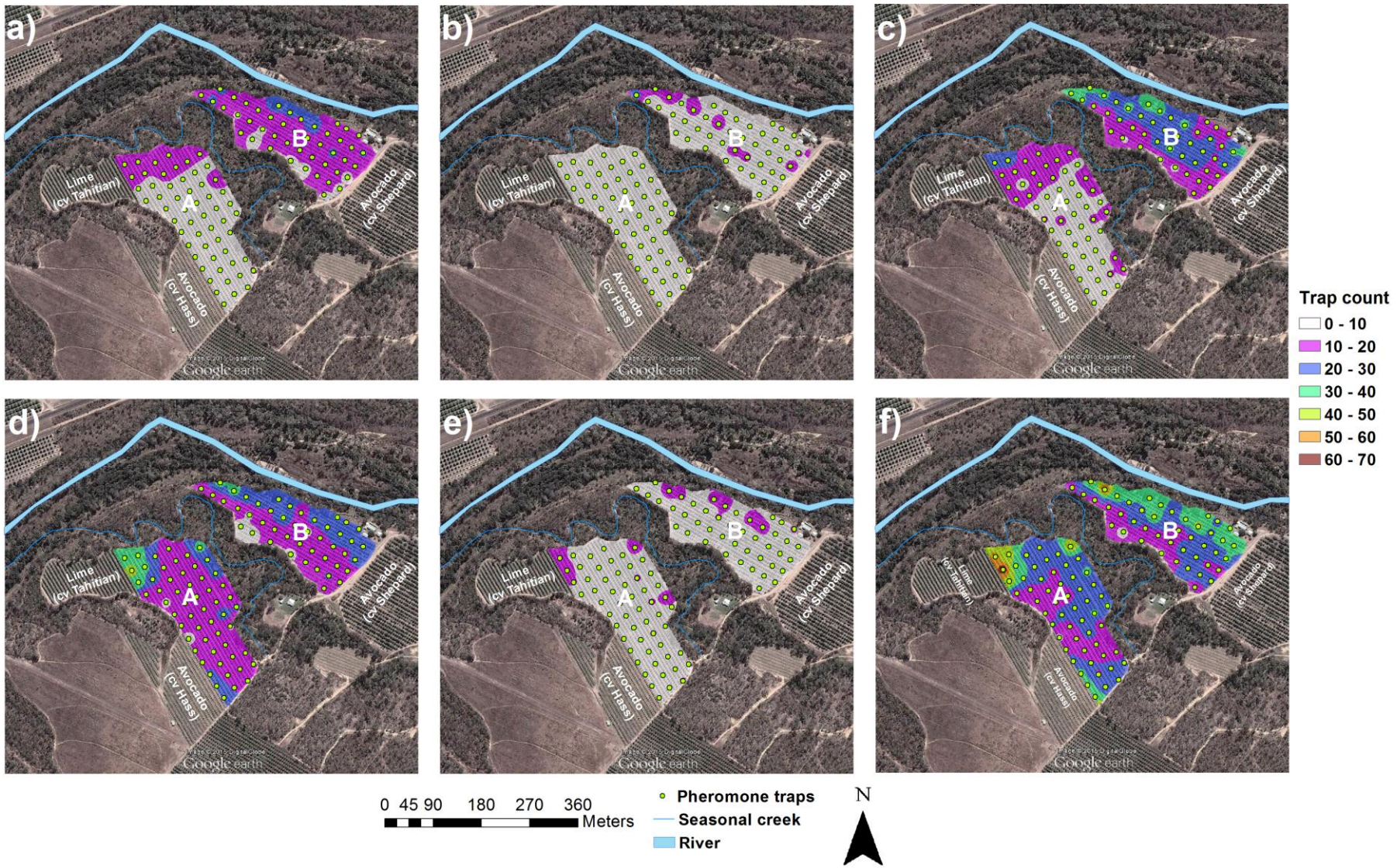

Figure 3.7. Spatial interpolations of total pheromone trap catch counts on field A and field B for *A. l. lutescens* adults during the 2013/2014 season (a), *A. l. lutescens* nymphs during the 2013/2014 season (b), total *A. l. lutescens* during 2013/2014 season (c), adult *A. l. lutescens* during the 2014/2015 season (d), nymph *A. l. lutescens* during the 2014/2015 season (e) and total *A. l. lutescens* during the 2014/2015 season.

Appendix 4. Potential applications of pheromone traps for IPM management of *Amblypelta lutescens lutescens* (Hemiptera: Coreidae) in avocado crops.

Methods

Study sites

Two avocado field sites were used in this study. Walkamin Research Station, Walkamin, Australia (-17.1377°, 145.4281°) was approximately 0.4 Ha and surrounded by mango trees *Mangifera indica* L. (c.v Kensington Pride and R2E2), lime trees *Citrus aurantifolia* L (c.v Tahitian) and dry sclerophyll forest. The avocado trees were three years old and going through their first major season of fruiting. No insecticides were applied during the field study.

The commercial orchard, near Mutchilba, Australia (-17.1198°, 145.2880°) was approximately 4.2 Ha and had dry sclerophyll forest surrounding the North, East and Western borders. The Walsh River flowed near the northeast boundary. The study was undertaken at the northern end of the block where higher population densities had previous been captured in pheromone traps (Lindsay 2016). Applications of β-cyfuthrin insecticides were applied using an air blast sprayer at recommended label concentrations to control *A. l. lutescens* populations every 10-14 days during the field study.

Pheromone traps

A prototype pheromone trap has been designed by the Department of Agriculture and Fisheries (DAF) in Queensland that uses the major chemical components emitted by male *A. l. lutescens* as an attractant lure. The trap consists of green twin-walled polypropylene sheeting (Corflute®) covered by double sided tape coated with an acrylic adhesive. Thin wire is used to secure the rubber septa containing the pheromone in a hole at the centre of the trap. Concentrations of pheromone components used is protected Intellectual Property. Pheromone traps were changed every fortnight due to UV degradation of the pheromone traps adhesive surface. Field trials had previously found the maximum attractiveness of the artificial pheromone to six weeks in the field (HAC Fay unpubl. 2011). Therefore, the pheromone septa in each pheromone trap were changed every six weeks.

Correlation between pheromone trap counts and avocado fruit damage

At all field sites, pheromone traps were distributed and inspected once a fortnight by inspecting both sites of the pheromone trap for any captured adult and nymph *A. l. lutescens*. Damage assessments were conducted once a fortnight by observing the surface of every tagged fruit for new *A. l. lutescens* feeding sites. New feeding sites were recorded and marked with a permanent marker to prevent them being counted in future damage assessments. At Walkamin Research Station, six pheromone traps were distributed on avocado trees approximately 30 m apart. Damage assessments on avocado fruit with *A. l. lutescens* feeding damage were also conducted on six trees containing pheromone traps (trap trees) and 24 trees that were 6 m away on the same row and between rows from trap trees (neighbour trees) (Figure 4.1) Ten avocado fruit were randomly selected on each damage assessment tree and the branches attached to the fruit were tagged with pink flagging tape. At the commercial orchard, nine pheromone traps were distributed approximately 36 m apart. Damage assessments were conducted in the nine traps trees, 18 neighbour trees and 11 trees that were 18 m away from trap trees (distant trees) (Figure 4.2). Ten avocado fruit were

again randomly selected per tree and branches attached to the fruit were tagged with pink flagging tape. Neighbour trees and distant trees were examined on the same row as trap trees but not between rows.

Damaged avocado fruit in trap trees vs non-trap trees

At the end of the field trial on correlations between pheromone trap counts and *A. l. lutescens* feeding damage, monitored fruit from trap trees and non-trap trees were harvested with fruit skin peeled off using vegetable peelers due to many feeding sites not being externally visible. At Walkamin Research Station, all tagged avocado fruit were harvested for an analysis of feeding damage in trap trees and neighbour trees. At the commercial orchard, half of the tagged fruit were harvested for an analysis of damage in trap trees, neighbour trees and distant trees. The proportion of avocado fruit with *A. l. lutescens* feeding damage and mean number of feeding sites per fruit was recorded.

Proportion of externally visible A. l. lutescens feeding sites

To determine the actual proportion of *A. l. lutescens* feeding sites externally visible compared the overall number of feeding sites. Externally visible *A. l. lutescens* feeding sites on avocado fruit harvested from both field sites were recorded before being peeled with a vegetable peeler and compared with the number of feeding sites visible after being peeled.

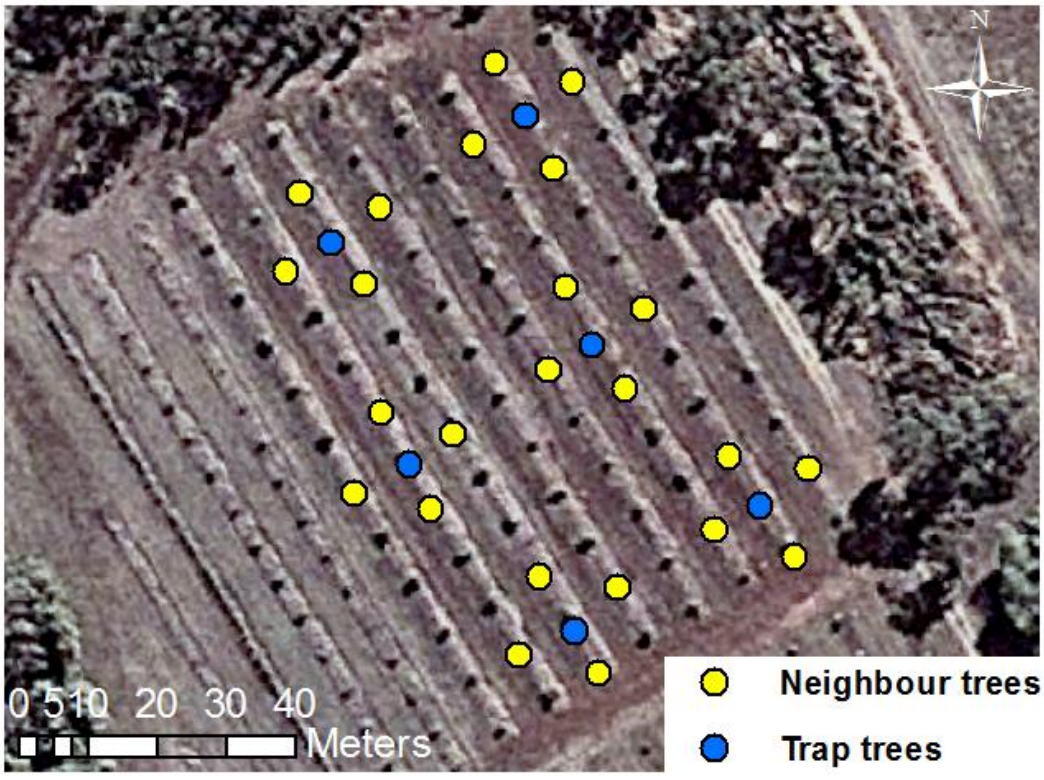

Figure 4.1. Walkamin Research Station field site with positions of trap trees and neighbour trees

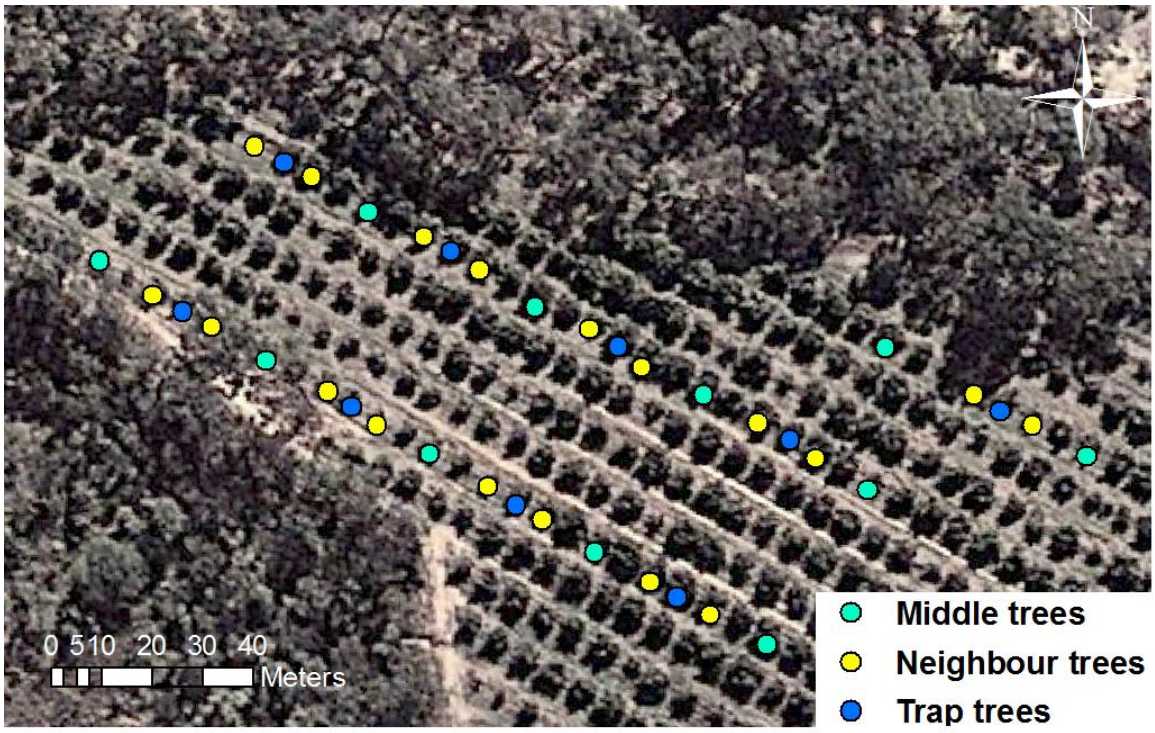

Figure 4.2. Commercial orchard field site with positions of trap trees, neighbour trees and distant trees

Statistical analysis

Data were analysed using GenStat 16 statistical package, version 16.1 (Genstat 16 committee 2013). Simple Linear Regression was used to explore correlations between fortnightly damage assessments on the proportion of avocado fruit damaged by *A. l. lutescens* and the mean number of *A. l. lutescens* adults and nymphs captured in pheromone traps. Correlations were explored between pheromone trap catch data and damage assessment data from trap trees and non-trap trees separately and combined. Generalised Linear Models (GLM) with a binomial distribution and logit link function were used analyse the proportion of avocado fruit with *A. l. lutescens* feeding damage in trees containing pheromone traps and trees 6 m or 18 m away from pheromone traps. Significant differences were analysed using Fishers unprotected Least Significant Difference (LSD) test. GLM's with a Poisson distribution and logarithm link function were used to analyse the mean number of feeding sites for each treatment.

Results

Correlations between pheromone trap counts and avocado fruit damage

Walkamin Research Station

There was a statistically significant correlation between the proportion of tagged avocado fruit with *A. l. lutescens* feeding damage on trap trees and the mean number of adult and nymph *A. l. lutescens* caught in pheromone traps per fortnight ($F_{1,8}=10.49$, $p=0.012$). The linear regression equation, $y=10.437x - 0.08871$ (Figure 4.1) predicts *A. l. lutescens* feeding damage to occur in avocado trees containing pheromone traps when mean fortnightly pheromone trap counts of *A. l.*

lutescens are above 0.008. There were no correlations between the proportion of tagged avocado fruit with *A. l. lutescens* feeding damage on neighbour trees and the mean number of adult and nymph *A. l. lutescens* caught in pheromone traps per fortnight (F1,8=0.22, p=0.651) and no correlations between the proportion of tagged avocado fruit with *A. l. lutescens* feeding damage on trap trees and neighbour trees combined (F1,8=0.02, p=0.882).

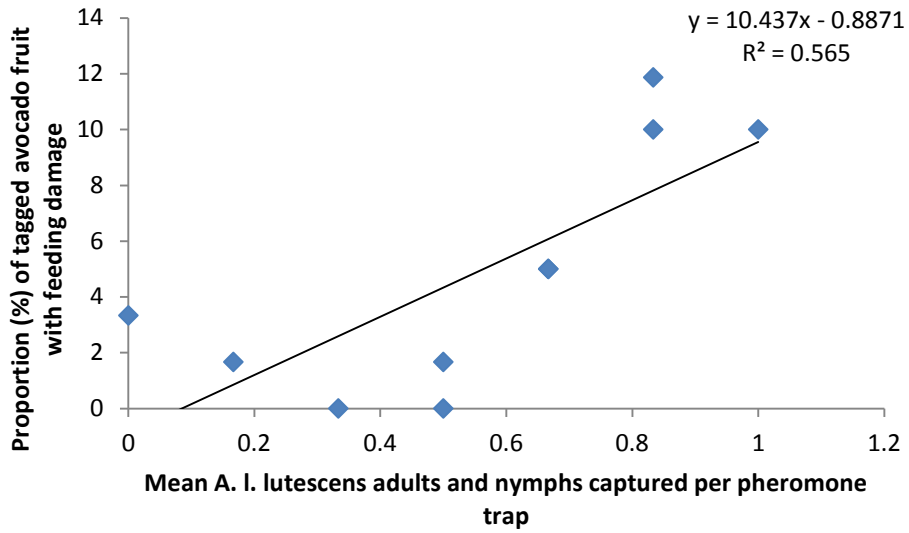

Figure 4.1. Mean *A. l. lutescens* adults and nymphs captured per pheromone trap versus the proportion of tagged avocado fruit with feeding damage caused by *A. l. lutescens* on avocado trees containing pheromone traps

Commercial orchard

There were no significant correlations between the proportion of tagged avocado fruit with *A. l. lutescens* feeding damage on trap trees (F1,4=0.32, p=0.600), feeding damage on neighbour trees and distant trees (F1,4=0.11, p=0.757) and feeding damage found on trap trees, neighbour trees and distant trees combined (F1,4=0.77, p=0.430).

Proportion of externally visible *A. l. lutescens* feeding sites

Only 21.64% and 26.15% of the total number of feeding sites were visible before skin peeling on fruit from Walkamin Research Station and the commercial orchard field sites.

Damaged avocado fruit in trap trees vs non-trap trees

Walkamin Research Station

There was no significant difference in the proportion of tagged avocado fruit with *A. l. lutescens* feeding damage in trap trees and neighbour trees F1,28=0.76, p=0.392 (Table 4.1) There were significantly more feeding sites per avocado fruit in trap trees compared to neighbour trees (F1,298=11.30, p<0.001) (Table 4.1).

Commercial orchard

There was a significantly higher proportion of tagged avocado fruit with *A. l. lutescens* feeding damage in trap trees compared to neighbour trees and distant trees $F_{2,35}=4.27$, $p=0.022$ (Table 4.1). An unprotected Fisher's LSD test indicated no significant difference in the proportion of tagged avocado fruit with *A. l. lutescens* feeding damage between neighbour trees and distant trees. There were also significantly more feeding sites per avocado fruit in trap trees compared to neighbour trees and distant trees $F_{2,187}=36.37$, $p<0.001$ (Table 4.1). An unprotected Fisher's LSD test indicated no significant difference in the mean number of feeding sites per avocado fruit between neighbour trees and distant trees.

Table 4.1. Mean (± SE) proportion of tagged avocado fruit with *A. l. lutescens* feeding damage and number of feeding sites per avocado fruit.

	% of fruit (±SE) with feeding damage		**Mean no. feeding sites (±SE) per avocado fruit**	
	Walkamin Research Station	**Commercial orchard**	**Walkamin Research Station**	**Commercial orchard**
Trap tree	60.0 ± 3.16 n=6	22.22 ± 6.53a n=9	5.32 ± 0.97a n=6	1.64 ± 0.27a n=9
6m away	41.74 ± 1.31 n=24	7.78 ± 2.97b n=18	2.39 ± 0.32b n=24	0.14 ± 0.05b n=18
18m away	-	3.64 ± 2.65b n=11	-	0.03 ± 0.04b n=11

Different letters within columns represent significant between treatments found using an unprotected Fisher's LSD ($p<0.05$).