

Final Report

Biology, Species and Genetic Diversity of Macadamia Lace Bugs

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Biology, Species and Genetic Diversity of Macadamia Lace Bugs – MC13008

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Summary

Agricultural systems worldwide increasingly face the emergence of novel pests. Within the last decade, one such pest became established in macadamia orchards in northern New South Wales and southern Queensland. The macadamia lace bug, *Ulonemia decoris* Drake, 1942 is a native to the region, but causes damage to macadamia flowers via feeding. This study provides the first comprehensive overview of the genus *Ulonemia* Drake and Poor, 1937 and its status in Australia. This study had three broad goals: 1) describe the relationship between *Ulonemia* and other lace bugs in Australia using morphology and genetics; 2) redefine *Ulonemia* using the results from part 1 and describe any new species; 3) examine the population genomics of *U. decoris* to determine the movement pattern of individuals between populations.

Four genes and 50 morphological characters were used to determine the relationships between *U. decoris* and other lace bugs. *Ulonemia* was found to be an artificial genus made up of 3 distinct groups, and although these groups are still closely related, they were distinct enough to call them separate genera. Two new genera, *Cercotingis* and *Proteatingis*, and six new species were described to account for these findings. *Ulonemia* was broken up to fit this new classification, with *U. decoris* transferred into *Cercotingis*; three other species of *Ulonemia* were transferred to *Proteatingis*. In addition, host plants for each of these species were compiled from records and observations; *C. decoris* has been found on *Macadamia* species as well as *Hicksbeachia pinnatifolia*, but no individuals were found on tallowwood or other common plants around orchards.

Understanding the lace bugs movement between orchards is critical in developing better control methods. Dispersal ability can provide valuable information on the geographic extent over which control must be coordinated, and corridors that facilitate movement can be found and blocked. Variable sites in the genome, called single-nucleotide polymorphisms (SNP), were obtained for 324 specimens of *C. decoris* across 11 localities in northern NSW. A second pestiferous species of lace bug, *P. howardi*, was also analysed, with 236 specimens from 8 localities in northern New South Wales and southeast Queensland. *Cercotingis decoris* exhibits minimal genetic differentiation over geographic distance, has a high dispersal rate between populations, low genetic diversity, and possible rapid reproduction due to parthenogenesis. In contrast, *P. howardi* exhibits significant genetic differentiation over geographic distance, a similarly high dispersal rate between orchards, and higher genetic diversity than *C. decoris*. These genetic, reproductive, and movement traits make both *C. decoris* and *P. howardi* difficult to manage, with orchard hygiene the best practice to prevent spread of individuals between orchards. There is also a need to monitor for other emergent pest species, because there are multiple lace bug lineages on plant species related to macadamia, with three of these lineages containing verified pests. In summary, optimal management of the species will likely require region-wide coordination.

Keywords

Macadamia; lace bug; pest management; population genetics; taxonomy; Ulonemia

Introduction

Agricultural systems worldwide increasingly struggle with the emergence of novel pests (Crowl et al. 2008). Often, these pests are alien species that have been either inadvertently or deliberately introduced into a new area (Pimentel et al. 2001). However, there are an increasing number of species that are pestiferous within their native range (Webber and Scott 2012). In Australia, there are several native taxa within the hemipteran family Tingidae that have become pests of various crops, including macadamias. The Tingidae is a family of cimicomorphan bugs, notable for their often-ornate ornamentation and lace-like texture on the wings, that comprise 260 genera and 2,124 species worldwide, with 56 genera and 147 species within Australia (Henry 2009). These tingids, or lace bugs, generally feed on leaves, with individual species mostly restricted to a narrow range of closely-related host plant taxa, though some species are more generalist in terms of host selection (Drake and Ruhoff 1965, Stonedahl, Dolling, and duHeaume 1992). One species of Australian tingid, the macadamia lace bug *Ulonemia decoris* Drake, 1942, has become a notorious pest in the Northern Rivers region of New South Wales over the last decade. This species has caused major economic losses in the macadamia nut industry (Huwier and Maddox 2007, Commens 2013) by feeding on and damaging macadamia flowers, preventing the development of nuts. Knowledge about this species and its relatives is sparse. This project grew from a push by macadamia growers to gather more information about these lace bugs. We were tasked to provide that information, consisting of a study of *U. decoris* and related species to uncover the diversity of macadamia lace bugs, and a population genetics study to determine the movement patterns of lace bugs in the Northern Rivers. The information generated by this study is critical for identifying pests in an orchard, potential novel infestations, and focusing management resources in an efficient manner.

Methodology

Systematics and phylogenetics

Macadamia lace bugs were collected from orchards across the Northern Rivers region of New South Wales. Most specimens were preserved in ethanol, though some specimens—mainly from orchards where lace bugs were in extreme abundance—were kept as dry material and mounted on paper points to serve as a reference collection. DNA was extracted from ethanol-preserved individuals, as well as other lace bug specimens to assess the taxonomy of the group. Four gene fragments were amplified from the resulting DNA for phylogenetic analysis: 16S rRNA, cytochrome c oxidase subunit I, 18S rRNA, and 28S rDNA. Additional lace bug sequences for taxa outside of Australia were obtained from Genbank; these were used as outgroups in the phylogenetic analysis. Species trees were constructed using Maximum Likelihood (RAxML) and Bayesian (MrBayes) methods. Morphological characters for a subset of the sequenced specimens of *Ulonemia* sensu lato species and closely allied taxa were quantified and combined with molecular sequence data for parsimony analysis.

Taxonomy

Over 700 specimens of Australian *Ulonemia* sensu lato were examined, along with habitus photos of holotype specimens for 5 Australian species and 4 extralimital taxa, in order to describe the diversity of the genus in Australia. Specimens that were physically examined were assigned a unique specimen identifier and were added to the Planetary Biodiversity Inventory Plant Bug (PBI) database (<http://research.amnh.org/pbi/heteropterasespeciespage/>). Thirteen morphometric characters (e.g. body length, body width, interocular distance) were recorded for each species of Australian *Ulonemia* sensu lato. The male genitalia were investigated for each species, sans *Cercotingis tasmaniensis* due to lack of male specimens. Habitus images were captured for both sexes of each species. Host plant data was recorded for each species, where available.

A list of all Australian lace bug genera associated with the plant family Proteaceae was assembled using records from the PBI database. Taxa that had >5 records from a proteaceous host, therefore signifying a host association and not an incidental record, were retained. Specimens of these taxa from the UNSW reference collection were examined and used to build a dichotomous key for species of potential concern to the macadamia industry.

Population genetics

Individuals of *U. decoris* were collected from 7 different sample localities located across the Northern Rivers region of New South Wales (NSW). These localities were chosen to maximise geographic coverage, with at least two pairs of localities separated by each of 300 m, 5 km, and 20 km distances to allow for isolation-by-distance (IBD) testing. Thirty individuals were collected from 5 localities, while 27 individuals were collected from 2 localities for a total of 204 individuals. Males, females, and nymphs were all used for analysis. Specimens were placed in individual wells of 96-well plates and sent to Diversity Arrays Technology (DArT) in Canberra for genotyping and single nucleotide polymorphism (SNP) extraction.

The dataset received from DArT contained 22,451 SNPs. The raw data were imported into RStudio using the package dartR; SNPs that fell below the quality threshold were removed from the dataset before further analysis, yielding 3,248 SNPs for *U. decoris*. Shannon's Information, $G''st$, Fis , and the number of populations present in the dataset were calculated using a Principle Coordinate Analysis (a process which uses similarity to create clusters of individuals) and the

program fastStructure (this process finds groups that have little to no deviation from Hardy-Weinberg Equilibrium).

Due to unexpected results in the above analyses, we determined further information was required to resolve some uncertainties in the dataset. To address this, 4 more localities from the Northern Rivers (each with 30 individuals of *U. decoris*) were sampled and added to the original dataset, resulting in 324 individuals collected from 11 localities. In addition, we decided that the second most prevalent pestiferous lace bug should also be analysed to provide a comparison between the two species. Individuals of the second species (*Proteatingis howardi*, described in Ryan Shofner's thesis) were collected from 8 sample sites across the Northern Rivers and southeast Queensland, with 30 individuals collected from each site—save one that had 26—resulting in 236 individuals total. These samples were sent to DArT for sequencing; 21,193 SNPs were amplified for *U. decoris*, and 30,358 SNPs were amplified for *P. howardi*. The raw datasets were processed identically to the original analysis, resulting in 6,132 SNPs for *U. decoris* and 5,521 SNPs for *P. howardi*. Shannon's Information, $G''st$, F_{IS} , and the number of populations present in the dataset were calculated for each species, using the program STRUCTURE, which operates in the same fashion as fastStructure, but with more accuracy.

Ecology/natural history

Several attempts were made to bring live specimens back to Sydney for observation in controlled settings, but all individuals perished en route or within a day of arrival. In addition, macadamia lace bugs have not been successfully reared in laboratory conditions due to their feeding behaviour, which requires live flowers still attached to their parent trees. Therefore, observations on lace bug life history were attempted *in situ*. Data were recorded both opportunistically during collection events, and by time-lapse photography of infested racemes (5-minute intervals over 12 hours).

The host plant range for all macadamia-associated lace bugs was determined by using a beating sheet and aspirator to collect specimens off of vegetation surrounding orchards, as well as a variety of native vegetation within natural reserves. Alternate hosts for macadamia-associated lace bugs were actively sought to determine whether additional plant species are acting as reservoirs for lace bugs, whence they can recolonise orchards. Host plant identities were confirmed by collecting a cutting of the vegetation upon which the bugs were found, and/or taking a photograph of identifiable characters of the host plant, which were then identified in the lab.

Insecticide trials

Various insecticides were tested for survivorship over a range of doses, with three replicates and per trial, and water as a control. Mortality was measured every 24 hours over a 3-day period. This work was conducted at the New South Wales Department of Primary Industries research station in Wollongbar; NSW DPI was sub-contracted to conduct these trials. See Appendix A for further details.

Outputs

- Ryan Shofner's PhD thesis - Taxonomy and phylogenetics of the genus *Ulonemia* Drake and Poor, 1937 (Heteroptera: Tingidae), with an emphasis on the population genetics of a pestiferous species. Completed August 30th, 2018
- Field assessment of insecticide efficacy on *Cercotingis decoris*
- Macadamia Lace Bug Fact Sheet (Version 2)
- Presentation to the Macadamia Consultants Meeting, Brisbane, June 2015
- Presentation to the Macadamia Consultants Meeting, Brisbane, June 2016
- Roundtable discussion on lace bug, Macadamia Industry Conference, Caloundra, October 2016
- Presentation to the Macadamia Consultants Meeting, Brisbane, June 2017
- Article for AMS Macadamia News Bulletin, April 2018
- Presentation to the IPM working group, Benowa, November 2018
- IPM Article for Macadamia Plant Protection Guide 2019/20

Outcomes

Systematics and phylogenetics

Currently, *Ulonemia* contains 16 species, 6 of which occur in Australia: *U. burckhardti*, *U. concava*, *U. decoris*, *U. leai*, *U. mjobergi*, and *U. plesia*. Two of these species were excluded from the Maximum Likelihood and Bayesian analyses: *U. concava*, which has not been collected since 1967, and *U. plesia*, as no specimens were available for sequencing. Parsimony analysis of molecular and morphological data yielded a large number of equally-likely trees, with many morphological characters shared between branches (homoplasy) (Shofner 2018, Figure 2.1, p. 35). In this study, the only clades with greater than 50% support at the generic level were *Epimixia* and *Nethersia*; both are definable by morphological characters. The genus *Ulonemia* sensu lato is highly unresolved in relation to the genera *Eriringis*, *Nethersia*, and *Tingis*. It is also unresolved in relation to several undescribed species that had putatively been assigned *Ulonemia*. As such, *Ulonemia* sensu lato does not have any morphological characters that unify the genus. *Ulonemia* sensu lato is paraphyletic in both Maximum Likelihood (Shofner 2018, Figure 2.2, p. 36) and Bayesian (Shofner 2018, Figure 2.3, p. 37) analyses, indicating that *Ulonemia* is not one genus, but rather three distinct, though closely related, genera.

Taxonomy

The genus *Ulonemia* is split into three genera: *Cercotingis*, *Proteatingis*, and *Ulonemia*. The macadamia lace bug, *U. decoris*, was transferred to a genus *Cercotingis*. Three new species of *Cercotingis* (*C. croajingolong*, *C. namadgi*, *C. tasmaniensis*) and 3 new species of *Proteatingis* (*P. astibosetes*, *P. howardi*, *P. minuta*) were described. There are 5 species of *Cercotingis* (*C. croajingolong*, *C. decoris*, *C. impensa*, *C. namadgi*, *C. tasmaniensis*), 6 species of the new genus *Proteatingis* (*P. astibosetes*, *P. burckhardti*, *P. howardi*, *P. minuta*, *P. mjobergi*, *P. plesia*), 11 species of *Ulonemia* (*U. angusta*, *U. aota*, *U. aptata*, *U. assamensis*, *U. dignata*, *U. electa*, *U. ermaea*, *U. jingae*, *U. leai*, *U. magna*, *U. malacciae*), though only *U. leai* occurs in Australia, and one taxon of uncertain placement, *U. concava* incertae sedis.

Of these lace bugs, *C. decoris*, *P. howardi*, *U. leai*, and *U. concava* incertae sedis occur on *Macadamia* species. All other *Cercotingis* and *Proteatingis* species use members of the Proteaceae as their hosts, and have the potential to be a future problem in macadamia orchards based on preferential host switching; the hosts for extralimital *Ulonemia* species are largely unrecorded.

Population genetics

The initial population genetics analysis of *C. decoris* indicates low genetic diversity (Shannon's Information 1H for all localities = 0.272) (Shofner 2018, Table 4.1, p. 171), a high inbreeding coefficient (mean F_{IS} across all localities = 0.165 ± 0.002) (Shofner 2018, Table 4.1, p. 171), and no significant genetic isolation by geographic distance (Mantel test for mutual information I , $r = 0.115$, $p = 0.236$, repetitions = 999) (Shofner 2018, Figure 4.2, p. 175) for the species across the Northern Rivers. No sample localities were found to be in Hardy-Weinberg equilibrium (HWE) across all SNP loci. The number of populations (K) in the dataset was calculated to be between 4 and 9, with $K = 4$ having the highest maximum marginal likelihood. Despite the fact that the algorithm used to determine K searches for clusters that have the smallest departure from HWE, positive within-cluster F_{IS} values were returned for 2 clusters at $K = 4$, which indicates a persistent excess of homozygous individuals in those two clusters. This result was highly unusual, so the dataset was expanded for a more in-depth analysis.

The second population genetics analysis of *C. decoris*, which combined the dataset from the first analysis with 4 additional sample localities and 120 additional individuals returned similar results to the initial analysis, with low genetic diversity ($^1H = 0.057$) (Appendix B, Table 1), a high inbreeding coefficient ($F_{IS} = 0.151$) (Appendix B, Table 1), and no significant genetic isolation by geographic distance (Mantel test for I , $r = 0.259$, $p = 0.051$, repetitions = 999) (Appendix B, Figure 1). The number of populations for this dataset was calculated at $K = 7$. Three of the K groupings generated by the analysis are comprised of individuals that have a high proportion of fixed alleles, with few to no private alleles. This indicates that these groupings are likely clonal lineages, as the SNP genotypes are identical within the groupings, and are complete subsets of the other K groupings, possessing no unique alleles.

As a comparison to *C. decoris*, we also analysed the population genetics of *P. howardi*. Genetic diversity was low across the entire sample ($^1H = 0.194$) (Appendix B, Table 2), though there was a marked difference between Queensland (mean $^1H = 0.160$) and New South Wales (mean $^1H = 0.214$) populations, with Queensland exhibiting lower genetic diversity. The inbreeding coefficient across the entire sample was high ($F_{IS} = 0.125$) (Appendix B, Table 2), indicating an excess of homozygotic individuals in the population, though again there was a contrast between Queensland (mean $F_{IS} = 0.046$) and New South Wales (mean $F_{IS} = 0.152$) samples; the low F_{IS} values for Queensland indicate that those localities are closer to HWE than the New South Wales localities. There is significant genetic isolation by geographic distance (Mantel test for I , $r = 0.799$, $p < 0.001$, repetitions = 999) (Appendix B, Figure 2). The number of populations for this dataset was calculated at $K = 2$, and represent a geographic split between Queensland and New South Wales. The Queensland group possess no private alleles and have lower genetic diversity compared to the New South Wales group, indicating that the Queensland genomes are a complete subset of the New South Wales genomes.

Ecology/natural history

Attempts to transplant live *C. decoris* to a lab setting for traditional life table studies were unsuccessful, with all specimens perishing during transportation. Locating populations of *C. decoris* with adequate population levels for observation has been extremely difficult due to pest management regimes at commercial macadamia orchards. Because of these difficulties, actual results of observation were minimal.

Both *C. decoris* and *P. howardi* cause damage to macadamia flower racemes; this was confirmed at orchards where either species occurred in isolation. However, *C. decoris* and *P. howardi*, both adults and nymphs, were observed coexisting in large numbers on the same macadamia flower on several occasions. Adults of both *C. decoris* and *P. howardi* were observed active on flowers at night, which included feeding by adults that remained stationary throughout daylight hours. Oviposition sites for *C. decoris* were observed on *Macadamia* sp. flowers, and include the peduncle, pedicel, and perianth.

The range of host plant species is narrow for both *C. decoris* and *P. howardi*. *Cercotingis decoris* was mainly collected from *Macadamia* spp., though large numbers have also been found on a macadamia-related proteaceous species *Hicksbeachia pinnatifolia* in the wild within the Northern Rivers. *Proteatingis howardi* has only been collected from *Macadamia* spp. No lace bugs were found in association with tallowwoods (*Eucalyptus microcorys*) or any other *Eucalyptus* species; indeed, no alternate hosts outside of the Proteaceae were found for any species of *Ulonemia* sensu lato.

Insecticide trials

Insecticide trials were completed successfully by NSW DPI. See Appendix A for the final results and report by NSW DPI.

Evaluation and discussion

The phylogenetic position of most Australian Tingidae still remain unresolved. The unique ornamentation of many members of the family obscures their phylogenetic relationships, as many morphological characters have apparently arisen multiple times independently, leading to problems with homoplasy. Few molecular studies of the family have been conducted to date, and more work needs to be done to fully understand the status of the family in Australia, and how it relates to the macadamia industry.

Between *Cercotingis*, *Proteatingis*, and *Ulonemia*, there are a total of 13 known species of lace bugs that depend on species of Proteaceae as their hosts. These occur in a wide variety of habitats, from mesic to semi-arid, and range from temperate Tasmania northwards along the east coast to the tropics, across the monsoonal grasslands and forests of northern Australia, to the Mediterranean climate of southwest Western Australia. Several species, *C. decoris* included, have been recorded from multiple genera of Proteaceae. This wide adaptability poses a risk for macadamia growers, as there is evidence to support that species within *Ulonemia* sensu lato are capable of switching hosts. For example, *U. leai*, which occurs in the wet tropics and the Atherton Tablelands, is a known pest of macadamias within this region. However, *Macadamia* sp. are not native to this area; all 4 species are restricted to southeast Queensland and northern New South Wales. Therefore, it can be deduced that *U. leai* has adapted to exploit the available resource presented by the proliferation of introduced *Macadamia* sp. in the Atherton district. This poses a risk to macadamia growers in other regions of Australia; there are at least two species of *Proteatingis* (*P. astibosetes* and *P. mjobergi*) that have been collected on *Grevillea* sp. in the macadamia growing regions of southeast Queensland and northern New South Wales. Given their proximity to macadamia orchards, these species may potentially infest orchards at some point in the future. The addition of novel pest species poses a risk to orchard management, as these additional species may react differently to current pest management regimes, especially if there is natural resistance to chemical treatment present in their genome which may be lacking in *C. decoris* and *P. howardi*.

Cercotingis decoris exhibits very little genetic differentiation between sample localities, and the low values for Shannon's mutual information combined with the lack of genetic isolation by geographic distance indicate that *C. decoris* disperses freely across the region. The presence of highly similar genotypes that represent subsets of the whole within the dataset indicates that some form of clonality or parthenogenesis is present in their mating system. Periodic parthenogenesis within an insect combines the advantages of parthenogenetic reproduction, which permits rapid increases in population in response to favourable environmental conditions, and sexual reproduction that allows recombination between genomes during outcrossing (White 1973, Sunnucks et al. 1997, Haack et al. 2000). Additionally, parthenogenesis is a frequent occurrence among pestiferous insects as an adaptation to monocultures in agricultural systems (Hoffmann et al. 2008). See Shofner 2018 pp. 182-184 for further discussion on pest management implications of these results.

The population genetics results for *P. howardi* show a stark contrast to the results for *C. decoris*. There is clear genetic isolation over geographic distance for *P. howardi*, though this is lacking in *C. decoris*. The genetic diversity in *P. howardi*, measured by 1H , is much higher than that of *C. decoris*. There does not appear to be any parthenogenic or clonal lineages within *P. howardi*; however, the Queensland genomes are a complete subset of the New South Wales genomes, indicating that the Queensland populations of this species were likely sourced from New South Wales, possibly as an historic introduction.

The excess homozygosity in a genome can be a sign of facultative or cyclical parthenogenetic reproduction (in the case of *C. decoris*) (Sunnucks et al. 1997), which is a potential adaptation to agricultural monocultures where particular clones can have elevated fitness across multiple generations (Hoffmann et al. 2008). It can also be a symptom of

extensive selective pressure through insecticide spraying or other control methods that can lead to bottleneck-like genetic signatures and loss of genetic diversity in a population (Silva-Brandão et al. 2015, De Luca et al. 2016).

Locating specimens of *C. decoris* in sufficient numbers for observation was exceedingly difficult. Managed commercial macadamia plantations excelled at controlling lace bug outbreaks over the duration of the study. Lace bug specimens could only be easily located by using beating sheets, at which point observation of natural behaviour was impossible. Attempts to re-establish individuals collected via beating sheet onto new flowers did not yield any results. The consequence of this meant that observations had to be conducted on “wild” populations—those occupying unsprayed trees.

Recommendations

- Increase orchard hygiene to control the spread of lace bugs by hitchhiking individuals via clothing and equipment
- Coordinate pest management over a wide geographic area to counter the rapid dispersal abilities of *C. decoris*
- Reduce monocultures, as these are often more susceptible to pests than systems with higher heterogeneity (Dalin et al. 2009, Guyot et al. 2016)
- Reduce reliance on pesticides, which can drive genetic selection towards resistance (Feyereisen et al. 2015)
- Adopt population genetics strategies to determine patterns of dispersal of pest species
- Target invasion pathways to efficiently use management resources

Scientific refereed publications

None to report.

Intellectual property/commercialisation

No commercial IP generated.

References

- Commens, R. 2013. Improved control of lacebug in macadamias. Horticulture Australia Ltd.
- Crowl, T. A., T. O. Crist, R. R. Parmenter, G. Belovsky, and A. E. Lugo. 2008. The spread of invasive species and infectious disease as drivers of ecosystem change. *Frontiers in Ecology and the Environment*. 6: 238–246.
- Dalin, P., O. Kindvall, and C. Björkman. 2009. Reduced population control of an insect pest in managed willow monocultures. *PLOS ONE*. 4: e5487.
- De Luca, D., G. Catanese, G. Procaccini, and G. Fiorito. 2016. *Octopus vulgaris* (Cuvier, 1797) in the Mediterranean Sea: Genetic Diversity and Population Structure. *PLOS ONE*. 11: e0149496.
- Drake, C. J., and F. A. Ruhoff. 1965. Lacebugs of the world. United States National Museum Bulletin. 243.
- Feyereisen, R., W. Dermauw, and T. Van Leeuwen. 2015. Genotype to phenotype, the molecular and physiological dimensions of resistance in arthropods. *Pesticide Biochemistry and Physiology*. 121: 61–77.
- Guyot, V., B. Castagnéyrol, A. Vialatte, M. Deconchat, and H. Jactel. 2016. Tree diversity reduces pest damage in mature forests across Europe. *Biology Letters*. 12: 20151037.
- Haack, L., J. C. Simon, J. P. Gauthier, M. Plantegenest, and C. A. Dedryver. 2000. Evidence for predominant clones in a cyclically parthenogenetic organism provided by combined demographic and genetic analyses. *Molecular Ecology*. 9: 2055–2066.
- Henry, T. J. 2009. Biodiversity of Heteroptera, pp. 223–263. In Footitt, R.G., Adler, P.H. (eds.), *Insect Biodiversity*. Wiley-Blackwell, Oxford, UK.
- Hoffmann, A. A., K. T. Reynolds, M. A. Nash, and A. R. Weeks. 2008. A high incidence of parthenogenesis in agricultural pests. *Proceedings of the Royal Society of London B: Biological Sciences*. 275: 2473–2481.
- Huwer, R., and C. Maddox. 2007. Lace bug – increasing incidences in NSW orchards.
- Pimentel, D., S. McNair, J. Janecka, J. Wightman, C. Simmonds, C. O’Connell, E. Wong, L. Russel, J. Zern, T. Aquino, and T. Tsomondo. 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment*. 84: 1–20.
- Shofner, R. 2018. Taxonomy and phylogenetics of the genus *Ulonemia* Drake and Poor, 1937 (Heteroptera: Tingidae), with an emphasis on the population genetics of a pestiferous species. Unpublished thesis: Biological, Earth & Environmental Sciences, Faculty of Science, UNSW, Sydney.
- Silva-Brandão, K. L., T. V. Santos, F. L. Cônsoli, and C. Omoto. 2015. Genetic Diversity and Structure of Brazilian Populations of *Diatraea saccharalis* (Lepidoptera: Crambidae): Implications for Pest Management. *Journal of Economic Entomology*. 108: 307–316.
- Stonedahl, G. M., W. R. Dolling, and G. J. duHeaume. 1992. Identification guide to common tingid pests of the world (Heteroptera: Tingidae). *Tropical Pest Management*. 38: 438–449.
- Sunnucks, P., P. J. De Barro, G. Lushai, N. MacLean, and D. Hales. 1997. Genetic structure of an aphid studied using

microsatellites: cyclic parthenogenesis, differentiated lineages and host specialization. *Molecular Ecology*. 6: 1059–1073.

Webber, B. L., and J. K. Scott. 2012. Rapid global change: implications for defining natives and aliens. *Global Ecology and Biogeography*. 21: 305–311.

White, M. J. D. 1973. *Animal cytology and evolution*. Cambridge University Press, London. 961 pp.

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Appendices

Appendix A – Report on DPI insecticide trials

Appendix B – Population genetics tables and figures

Appendix C – Macadamia Lace Bug Fact sheet (Version 2)

Appendix D – Article for AMS Macadamia News Bulletin

Milestone Report

Project code: MC13008

Project title: Biology, species and genetic diversity of Macadamia lace bugs

Milestone number: 109

Milestone due date: 31/05/2017

Research provider: University of New South Wales (UNSW)

Project leader: Gerry Cassis

Report author: Craig Maddox and Ruth Huwer

Milestone description: Laboratory work and insecticide trials.

Milestone achievement criteria: Report on genotypes identified to date. Report on 2016-17 insecticide trials.

Research and development (R&D) projects: levy funding

This project has been funded by Horticulture Innovation Australia Limited using the research and development Macadamia levy and funds from the Australian Government.

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Summary

This is a single spray application field test; the result is based on the comparison of *Ulonemia decoris* populations still active after 7 days on the treated macadamia racemes. Of the new options tested, ONLY Transform® at a 1 ml/L rate and Talstar® at a 0.5 ml/L rate are performing better than the registered treatments (Diazinon or Trichlorfon).

The natural pyrethrin (Pyganic®) at a 2 ml/L rate and the Bayer DC 143 at a 0.15 ml/L rate were reinfested with lace bugs by day 7, and performed no better than the control.

Sero X is effective at a 20 ml/L rate, which equates to 35-50 L/Ha on large trees, depending on the volume sprayed into each canopy. The Sero X treatment is the best “soft option” we have seen at a 20ml/L rate. It is not known whether Sero X is phytotoxic, or if it is effective at a lower rate. This should be investigated further.

The key risk is the Australian Pesticides and Veterinary Medicines Authority (APVMA) restricting use of chemistry that is effective, thus leaving the industry vulnerable to losses from lace bug. The new chemistries used on other pests in the industry will need to be tested for impact on lace bug as the new spray program is developed, and hopefully viable biological options are found.

Milestone achievements

Genotypes Identified

See milestone report from UNSW.

Report on 2016-17 Insecticide Trials

Methodology

Test compounds tried were sulfoxaflor (Transform), Bayer DC143, Bayer DC146, DC099, BAS 450001 (carboxamide), SeroX®, bifenthrin (i.e. Talstar), pyrethrin (i.e. Pyganic), and cyantraniliprole (Exirel) against the industry standard treatments of trichlorfon (i.e. Lepidex) and diazinon. Ideally, when a treatment is applied that has a fast half-life (i.e. half-life less than 1 day, as for natural pyrethrin) you can test the efficacy of a product against the pest by sampling the racemes on Day 3 after application. For an investigation of the residual effect as well as the “knockdown” a product has, a sampling 7 days after application is preferred because adult lace bugs originating away from the sprayed racemes can re-infest treated racemes within days. In addition, fresh young nymphs will be apparent if sampling is done 7 days after application. This can be extended to 14 or 21 days after application to investigate if the whole flowering period can be protected with a single application as has been done in previous work (NSW DPI 2009-2013 HAL MC06021 pp98-127 2011; MC11006); however, we did not investigate this during this milestone.

Pre-treatment *Ulonemia decoris* population levels: There is some level of resistance to *U. decoris* in some macadamia varieties. Therefore, the ideal method of application involves applying each chemical to flowers of the same or similar variety within each replicate. Racemes infested with *U. decoris* were collected from areas around the CTH Alstonville site on July 11th, 2016, and these were placed onto racemes of cv849 and cv246 trees, which are highly susceptible to the pest (Figure 1). Treated and untreated samples were examined under magnification and the population per raceme calculated (Table 1). However, as the population of *U. decoris* was reaching a treatable level, a significant storm (120 km/hr wind and 75 mm rain over about 5 hours) occurred on August 3rd, 2016, which impacted *U. decoris* population levels. The result was a 50% reduction of potential test plots and a reduction to only two replicates per treatment.

Application: Products were mixed 1 hour prior to application using A grade Blau® brand volumetric glassware. Test formulations were obtained from a variety of sources (some provided by manufacturers and some commercial formulations (Table 2)). These were used to make 250 ml stock solutions with demineralized water with a 0.1 ml/L addition of the spreader-sticker Designer® in each mixture at the rates listed (Table 2). Individual plastic misters set to fine misting and calibrated to deliver 0.5 mls per application were used to cover each raceme from 4 directions (2 mls applied to each raceme in fine mist). At least ten replicate racemes were treated in an area within the canopy where lace bug activity could be seen on the florets (Figures 1 and 3), and each branch was tagged with flagging tape and labelled by the product applied.

Evaluation: Racemes were collected 7 days after the application and all estimates of macadamia lace bug numbers were made under 15X magnification to determine the presence of live and dead nymphs or adults. The counts were transcribed to Microsoft Excel®, where means and standard errors were generated for the treatment comparison.



Figure 1: Transplanting *Ulonemia decoris* infested racemes from the Germplasm block (tied to branch between fresh racemes) into the entomology block at CTH Alstonville NSW to overcome the varietal differences and enhance the infestation before treatment.



Figure 2: Treatment of all racemes hanging off the branch around the labelled marking tape, each raceme received a 2 ml misting on August 8, 2016. The trees were revisited 7 days later on August 15th, 2016, and ten or more racemes were picked and put in sealed labelled paper bags and examined at 15 X magnification under a Zeiss stage microscope at Entomology laboratories at Wollongbar Primary Industries Institute.



Figure 3: *Ulonemia decoris* (macadamia lace bug) was present in all life cycle stages on untreated control racemes 1 day after treatment on August 9th, 2016. Each raceme is scored for presence or absence of nymphs and adults (live or dead), lace bug damage, and cast skins at 15X magnification under a Zeiss stage microscope.

Results

The two replicate regions had vastly different levels of *Ulonemia decoris* present, and in the CTH Entomology block every treatment was better than the control, which maintained a minor infestation (Table 2). In the accession block the higher population showed a better separation among treatments. Only the bifenthrin and sulfoxaflor at 1 ml/L treatments were superior to the registered treatments of trichlorfon or diazinon (Table 2). The Sero X[®] treatment also gave a good result (Table 2). As expected, the natural pyrethrin (Pyganic[®]) was showing re-infestation by 7 days, as was the experimental compound DC 143 (Table 2). The other treatments were not as effective as the currently registered options but better than the control (Table 2).

Phytotoxicity of the applications was also examined until August 29th, 2016, at which point the entire farm was treated to ensure nut-set in the coming season for other experiments. At that stage, the control and treatments of Pyganic[®], DC143 and DC146 were showing limited nut set and more lace bug activity. No poor flower growth was observed for any of the treatments to that point.

Table 1. Populations of *Ulonemia decoris* (MLB) on racemes prior to the application of pesticides. Populations from Macadamia Germplasm orchard were used to seed areas of florets in the CTH Entomology plot on July 7th, 2016. Storm and rain affected the MLB population on August 3rd, 2016 (75 mls and 120 km/hr winds) and we were only able to salvage two areas with pest activity to treat.

Date	Plot	Racemes	Total MLB	Live MLB nymphs	Live MLB adults	Total MLB/raceme	Standard error
11/07/2016	Germplasm block	47	160	140	20	3.4	0.6
8/08/2016	Accession block	10	84	69	10	8.4	3.3
8/08/2016	CTH Entomology block	12	19	9	10	1.6	0.6

Table 2. Comparison of the population levels of *Ulonemia decoris* (MLB) 7 days after application of 2 ml misting per raceme of the listed pesticide formulations at the specified rates. Treatments were applied on August 8th, 2016 and counted under 15X magnification on stage microscopes on August 15th and 16th, 2016 (Day7).

Code	Chemical	Formulation	Rate mls/L	Accession Block n=11 racemes		Entomology Block n=11 racemes	
				Live MLB/ raceme	Standard error	Live MLB/ raceme	Standard error
1	Control	water	0	3.9*	0.6	0.9*	0.3
2	Sulfoxaflor	Transform® 240gm/L	1	0.0	0.0	0.0	0.0
3	Sulfoxaflor	Transform® 240gm/L	0.4	1.5*	1.1	0.0	0.0
4	Trichlorfon	Lepidex® 500EC	2	0.2	0.2	0.0	0.0
5	Carboxamide	BAS 450001 100Sc	0.5	0.4	0.3	0.0	0.0
6	DC143	***	0.15	3.0*	0.6	0.0	0.0
7	DC146	***	0.3gm	1.1	0.5	0.0	0.0
8	DC099	***	0.6	1.2	0.7	0.1	0.1
9	Cyantraniliprole	Exirel® 100Ec	1	0.8	0.5	0.0	0.0
10	<i>Clitoria</i> extract	Sero X® 400g/L	20	0.1	0.1	0.0	0.0
11	Pyrethrin	Pyganic® 13gms/L	2	3.8*	1.1	0.0	0.0
12	Diazinon	Country® 800gm/L	1.25	0.2	0.1	0.0	0.0
13	Bifenthrin	Talstar® 80g/L	0.5	0.0	0.0	0.0	0.0
Pre Treatment				7.9	3.5	1.6	0.6

*Treatments not different using z test

Outputs

Field assessment of insecticide efficacy on *Ulonemia decoris*.

Refereed scientific publications

None to report.

Outcomes

Of the new options only Transform® at the 1 ml/L rate and Talstar® @ the 0.5 ml/L rate are performing better than the registered treatments of diazinon or trichlorfon.

Sero X® is effective at the 20 ml/L rate.

The natural pyrethrin (Pyganic®) at the 2 ml/L rate and DC 143 at the 0.15 ml/L rate are reinfested with *U. decoris* by day 7 and are therefore no better than the control.

Intellectual property, commercialisation and confidentiality

Source of experimental chemicals need to be kept confidential.

Issues and risks

The Sero X® treatment is the best “soft option” we have seen at the 20 ml/L rate. There is no information on the phytotoxicity of the product that we are aware of. Also, a minimum rate of effectiveness will need to be established. Both should be further investigated on populations of *U. decoris* as they arise. This will be done at the onset of flower 2017, though this is not part of this project. The key risk is the APVMA restricting use of chemistry that is effective, thus leaving the industry vulnerable to the losses from macadamia lace bug. The new chemistries used on other pests in the industry will need to be tested for impact on macadamia lace bug as the new spray program is developed and hopefully viable biological options are found.

Other information

With extensive management of *Sigastus* weevil and *Amblypelta nitida* (fruitspotting bug (FSB)) occurring in the Northern Rivers district in response to high prices for nut-in-shell, the actual incidence of *Ulonemia decoris* (macadamia lace bug) fell dramatically in 2013/14 and further in 2014/15. The beta-cyfluthrin, acephate, and methidathion applied for *Sigastus* weevil and *A. nitida* have impacted heavily on the lace bug population.

Not many growers were prepared to risk crop loss due to pest activity at flowering, and most conventional growers opted to use diazinon pre-flowering, rather than waiting to use trichlorfon during flowering, and record crops have been reported in areas that were lace bug infested (AMS Benchmarking 2014-16). There has also been a dramatic drop in the number of organic growers, and those remaining have been applying natural pyrethrin (up to 4 applications) fortnightly to ensure good nut-set. Good spray coverage in an orchard and good spray timing have been very effective. Buffer zones around houses and areas where orchards are unsprayed still have problems with *U. decoris* infestations.

Field trials for macadamia lace bug activity at CTH were planned to take place in September 2015. We need background levels of activity to be detectable on at least 10 racemes per treatment before we apply the treatment to have any chance of measuring a significant drop in survival rates on the racemes 3-7 days after application. Some activity was present in several areas in late August 2015, but this activity was restricted to isolated racemes. Bayer Crop Science was also interested in conducting a separate trial and was planning to conduct another trial in another plot as well when we felt the numbers were high enough. By mid-September things were building but a large and unusually intense rain event (>100mls over four days 19-

22 September 2015) created enough stem flow to remove the harbouring adult lace bugs and ruined at least a week of open flowers in a lot of the macadamias around Lismore. Both our trial and the Bayer Crop Science spray trials were put on hold until higher population levels of lace bug could be found.

We were planning to repeat the experiment on the out-of-season flowering in April 2016, but pest numbers were again too low. A build-up of lace bug numbers resulting from a lack of spraying allowed us to conduct the field trial during the main flowering in spring 2016. At CTH Alstonville we were able to conduct trials with unregistered chemicals without having to compensate for lost crop, with two areas that are maintained as untreated plots to facilitate work on flower pests.

Appendices

None.

Table 1. Summary statistics for *Cercotising decoris* genotypic data. For each locality, top row = mean, bottom row = standard error; coordinates are in WGS84. N = number of samples; Lat = latitude; Lon = longitude; H_o = observed heterozygosity; μH_e = unbiased expected heterozygosity = $(2N / (2N-1)) * H_e$; F_{IS} = Fixation Index = $(H_e - H_o) / H_e = 1 - (H_o / H_e)$; 1H = Shannon Information ($q = 1$ α -diversity).

Locality	N	Lat	Lon	H_o	μH_e	F_{IS}	1H
BH1	29.826	-28.73000	153.54420	0.064	0.072	0.080	0.114
	0.010			0.002	0.002	0.004	0.003
BH2	26.432	-28.73132	153.54176	0.076	0.093	0.141	0.157
	0.011			0.002	0.002	0.004	0.002
CR1	29.671	-28.86740	153.40280	0.074	0.089	0.139	0.148
	0.010			0.002	0.002	0.004	0.003
CR2	29.389	-28.86670	153.40177	0.076	0.092	0.139	0.154
	0.012			0.002	0.002	0.004	0.003
DPI	29.731	-28.8477	153.45557	0.071	0.088	0.165	0.145
	0.010			0.002	0.002	0.004	0.003
DR1	29.716	-28.59489	153.39293	0.078	0.095	0.143	0.158
	0.010			0.002	0.002	0.004	0.003
GW1	26.603	-28.67940	153.30740	0.073	0.094	0.176	0.154
	0.012			0.002	0.002	0.004	0.003
HP1	29.632	-28.58972	153.39772	0.081	0.101	0.164	0.163
	0.013			0.002	0.002	0.004	0.003
PK1	29.732	-28.59489	153.39293	0.079	0.092	0.128	0.149
	0.011			0.002	0.002	0.004	0.003
PW1	29.605	-28.83400	153.36420	0.073	0.084	0.105	0.135
	0.011			0.002	0.002	0.004	0.003
WK1	28.475	-28.64929	153.32461	0.074	0.096	0.195	0.164
	0.019			0.001	0.002	0.004	0.002
Total	29.846			0.030	0.037	0.151	0.057
	0.003			0.000	0.000	0.001	0.001

Table 2. Summary statistics for *Proteatingis howardi* genotypic data. For each locality, top row = mean, bottom row = standard error; coordinates are in WGS84. N = number of samples; Lat = latitude; Lon = longitude; H_o = observed heterozygosity; μH_e = unbiased expected heterozygosity = $(2N / (2N-1)) * H_e$; F_{IS} = Fixation Index = $(H_e - H_o) / H_e = 1 - (H_o / H_e)$; 1H = Shannon Information ($q = 1$ α -diversity).

Locality	N	Lat	Lon	H_o	μH_e	F_{IS}	1H
AZ1	29.740	-26.83775	152.96323	0.106	0.102	-0.002	0.153
	0.012			0.003	0.002	0.005	0.003
AZ2	29.674	-26.83413	152.96691	0.097	0.105	0.073	0.158
	0.013			0.002	0.002	0.005	0.003
BH1	29.563	-28.73000	153.54420	0.115	0.137	0.145	0.216
	0.012			0.002	0.002	0.004	0.003
DR1	25.668	-28.59489	153.39293	0.113	0.135	0.138	0.214
	0.010			0.002	0.002	0.004	0.003
JB1	29.617	-26.85157	152.85448	0.103	0.111	0.068	0.168
	0.014			0.002	0.002	0.004	0.003
PK1	29.464	-28.59489	153.39293	0.105	0.134	0.186	0.216
	0.015			0.002	0.002	0.004	0.003
PW1	29.583	-28.83400	153.36420	0.113	0.136	0.147	0.213
	0.012			0.002	0.002	0.004	0.003
VP1	29.584	-28.90111	153.40998	0.113	0.135	0.145	0.213
	0.012			0.002	0.002	0.004	0.003
Total	29.112			0.108	0.124	0.125	0.194
	0.008			0.001	0.001	0.002	0.001

Genetic isolation by geographic distance

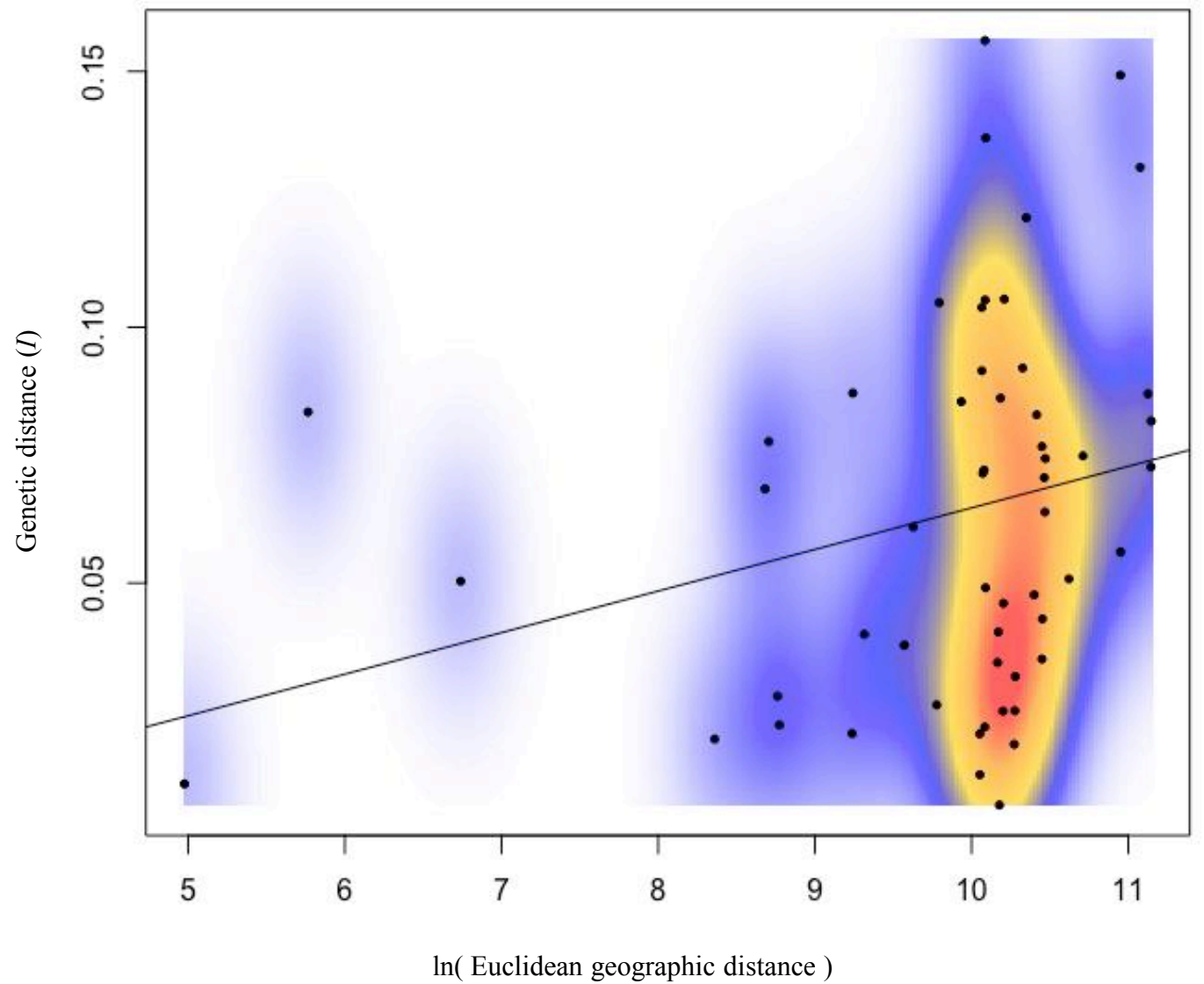


Figure 1. Genetic isolation by distance plot for *Cercotising decoris*. Genetic distance is Shannon's mutual information I , geographic distance is the natural log of the Euclidean distance between geographic coordinates.

Genetic isolation by geographic distance

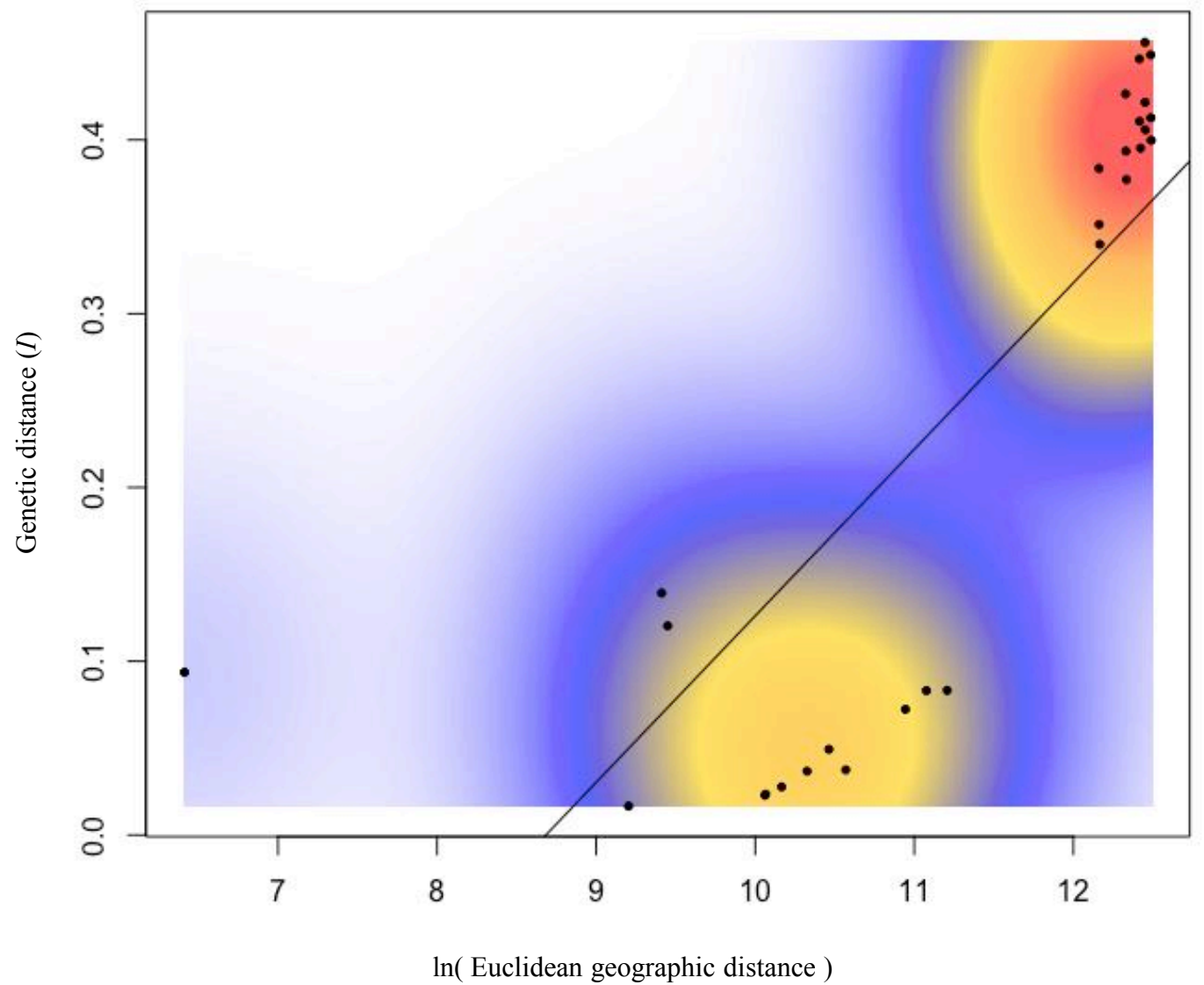


Figure 1. Genetic isolation by distance plot for *Proteatingis howardi*. Genetic distance is Shannon's mutual information I , geographic distance is the natural log of the Euclidean distance between geographic coordinates.

What is a lace bug?

Lace bugs are small insects, usually 3 to 4 mm in length; they comprise the family Tingidae within the order Hemiptera (the true bugs). Lace bugs have piercing/sucking mouthparts, which they use to pierce plant tissues to feed on sap. Lace bugs get their name from the pattern of cells on their hemelytra and thorax, and the complex ornamentation of some species. Their small size makes them hard to detect with the naked eye, but they are readily distinguishable under a hand lens or microscope.

Life history

Most lace bugs feed on the leaves of plants, though some species feed on flowers. Lace bugs can cause damage to plant tissues through their feeding. Each lace bug species typically feeds on very specific plants; the plant species, or hosts, are often closely related to each other, like *Grevillea* and *Macadamia*.

Eggs are laid within plant tissues, and nymphs emerge within a few days. The nymphs remain near where they hatch, and feed in the same manner as adults. However, nymphs lack wings, and undergo 5 stages, called instars, before they become adults. Adults are able to fly, and disperse readily between populations. Recent genetic studies indicate that adults are able to disperse considerable distances, with populations up to 20 km apart being highly related to one another. This ability enables lace bugs to rapidly recolonise areas where they have been eradicated.

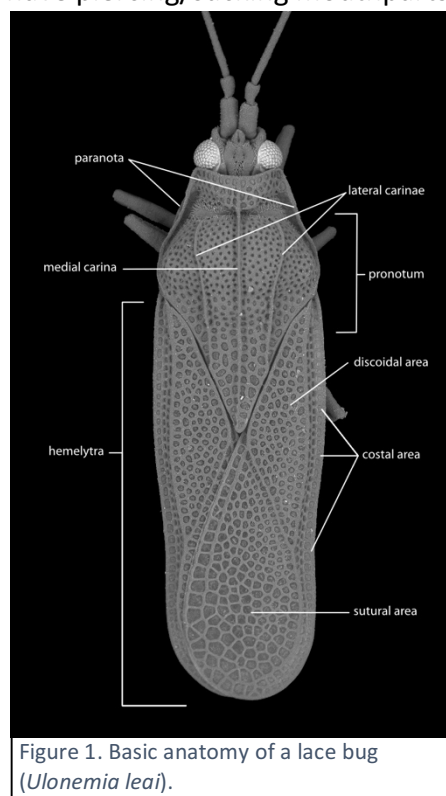


Figure 1. Basic anatomy of a lace bug (*Ulonemia lei*).

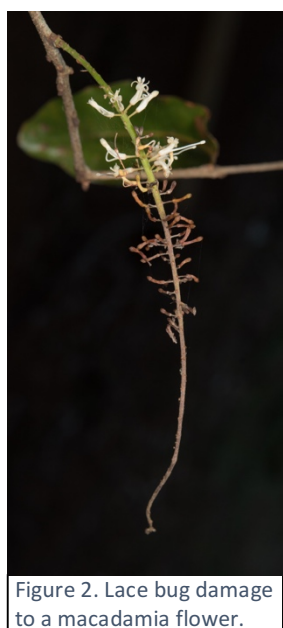


Figure 2. Lace bug damage to a macadamia flower.

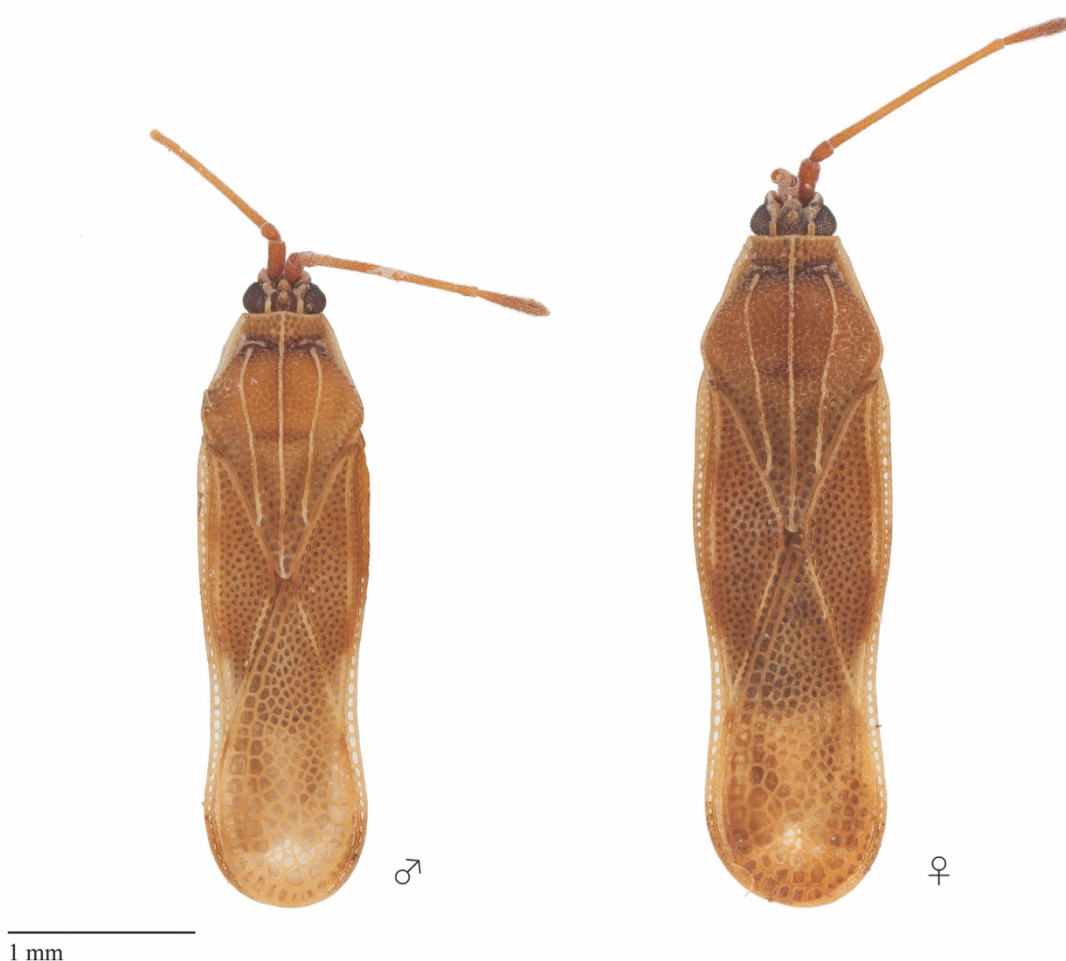
Impact on macadamias

Several species of lace bugs in the genus *Ulonemia*, which are native to Australia, have become important agricultural pests and are responsible for major economic losses within the macadamia industry. They feed on and damage macadamia flowers, and their populations can build up rapidly if left unchecked. Recent changes to industry regulations and lack of knowledge on these insects has led to difficulties with their control. The varying life histories and host plants between species means that proper identification is crucial so that a proper management regime can be enacted.

Identification

The next section serves as an identification guide to lace bugs found on macadamias in Australia, as well as an additional related species that occurs in macadamia growing regions and may have pest potential.

Ulonemia concava



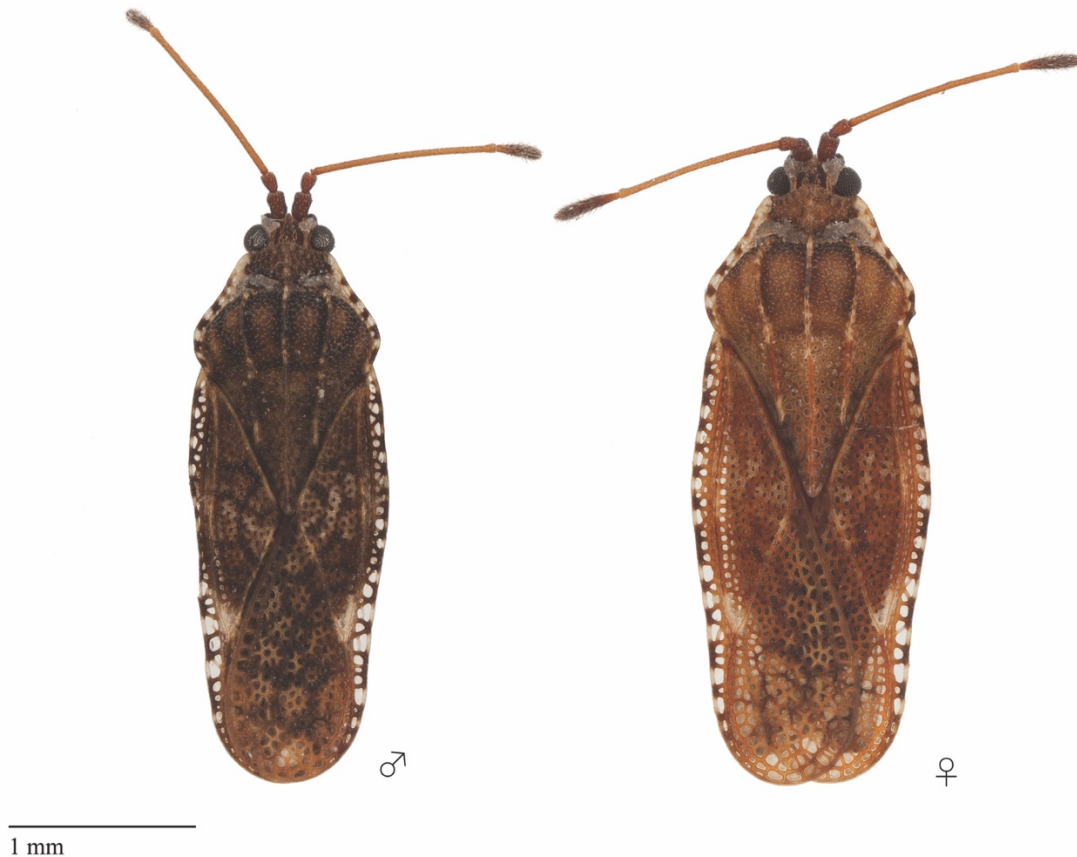
- 3.4-3.9 mm in length
- Dark eyes
- “Narrow-waisted”: wings narrow and then flare out again to approximately the width of the body
- Paranota appear fused to the thorax
- Front of thorax mostly straight
- Found on macadamias
- Previously collected from Maleny (likely occurs in nearby regions)
- Last confirmed sighting in 1967

Ulonemia decoris



- 3.1-3.6 mm in length
- Red eyes
- “Narrow-waisted”: wings narrow and then flare out again to approximately the width of the body
- Paranota raised away from thorax, with many small cells visible
- Wings with large inverted heart-shaped dark brown or black marking
- Found on macadamias
- Major pest that can cause considerable nut loss
- Southeast Queensland from Gympie to Mt. Glorious, Beerwah, south to Springbrook National Park; North-eastern New South Wales from the Border Ranges, across the Northern Rivers region, south to at least Nambucca

Ulonemia new species



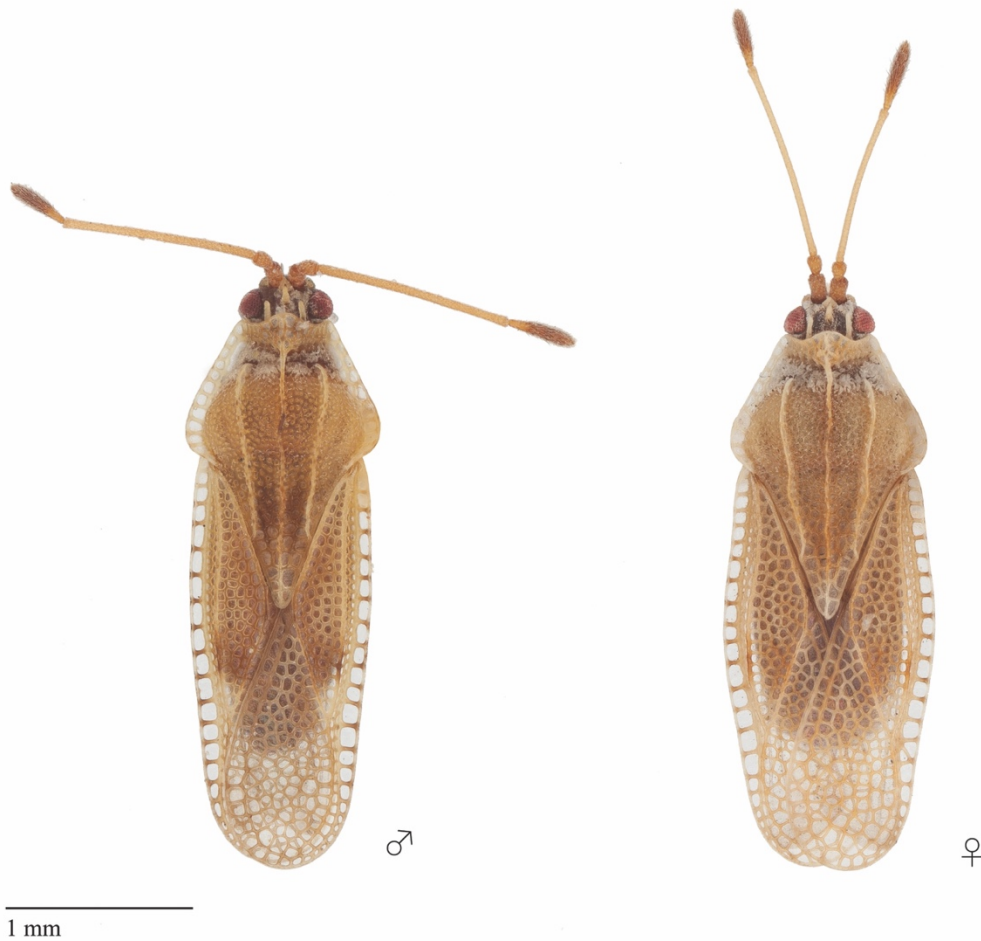
- 2.8-3.4 mm in length
- Dark eyes
- Reddish-brown to black reticulate pattern on hemelytra
- Cells in costal area irregularly shaped (rather than roughly rectangular)
- Wing ends narrower than the width of the body
- Paranota raised away from thorax, 1-2 cell rows visible
- Found on macadamias
- Major pest that can cause considerable nut loss
- Southeast Queensland from Gympie to Mt. Glorious, Beerwah, south to Springbrook National Park, a single specimen is known from Bundaberg; North-eastern New South Wales from the Border Ranges, and across the Northern Rivers region

Ulonemia leai



- 3.1-3.6 mm in length
- Red eyes
- Wings ends narrower than body, less “narrow-waisted” than *U. concava* or *U. decoris*
- Paranota almost appearing fused with thorax, but a single row of cells is visible along their entire length
- Front of thorax noticeably curved
- Found on macadamias and silky oak (*Grevillea robusta*)
- Major pest that can cause considerable nut loss
- Wet tropics and Atherton Tableland in Queensland; recently collected 1,400 km south near Springbrook National Park, likely widespread across eastern Queensland

Ulonemia mjobergi



- 3.0-3.5 mm in length
- Red eyes
- Wings ends narrower than body
- Paranota raised away from thorax, single row of cells clearly visible
- Front of thorax noticeably curved forward in the middle
- Cells of costal region large and square-like
- Found on various *Grevillea* species
- Not yet recorded from macadamias, but is closely related to pest species, and occurs near macadamia growing areas
- From the Kimberley in northern Western Australia, across the Top End into northern Queensland including Cape York, south to around Mareeba, isolated record from White Mountains National Park; recent record from the Glasshouse Mountains in south-eastern Queensland

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An overview of the Macadamia Lace Bug project

If there's a boogeyman for Australian macadamia growers, it's undoubtedly a tiny, ornamented insect. Like the boogeyman, lace bugs have the tendency to pop up when you least expect it, and can rapidly increase in population and cause startlingly large amounts of damage to macadamia flowers. Yet, very little was actually known about these bugs. Through a partnership between UNSW Sydney, Horticulture Innovation Australia Limited, the Australian Macadamia Society, and NSW DPI, we have been investigating some of the basic aspects of lace bugs in order to provide a foundation on which management and research programs can be built.

What's a lace bug, exactly?

Lace bugs are small insects, usually 3 to 4 mm in length; they comprise the family Tingidae within the order Hemiptera (the true bugs). Lace bugs have piercing/sucking mouthparts, which they use to pierce plant tissues to feed on sap. Lace bugs get their name from the pattern of cells on their wings (hemelytra), which look like sewing lace in some species. Their small size makes them hard to detect with the naked eye, but they are readily distinguishable under a hand lens or microscope.

Within Australia, lace bugs have been reported on macadamias from NSW from Nambucca, north to the Northern Rivers region, and in QLD from the NSW border to Gympie, as well as the Atherton Tableland. So far, they appear to be absent from Bundaberg apart from a few single specimens. These macadamia-associated lace bugs are all members of the genus *Ulonemia*, which has a native distribution from Australia through the Indo-Pacific and China. Australia has around 13 species of *Ulonemia*, 6 of which are still undergoing formal description. Of these, 4 have been found in large numbers on macadamias.

Lace bugs are generally host-specific; that is, each species only uses a particular type of plant for feeding. Members of the genus *Ulonemia* have thus far only been recorded from plants in the family Proteaceae: *Macadamia*, *Grevillea*, *Hakea*, and a few others. While some species of *Ulonemia* have been recorded using several different types of plants within the Proteaceae, the high amount of host specificity means that it is unlikely that lace bugs use other unrelated hosts, such as tallowwood (Myrtaceae).

Macadamia-associated lace bugs

The 4 species of lace bug known to occur on macadamias are *Ulonemia concava*, *U. decoris*, *U. leai*, and a yet-to-be-described species ('*Ulonemia* sp. nov.') within the same genus. *Ulonemia decoris* and *U. sp. nov.* are the predominant pestiferous species in the Northern Rivers and southern Queensland; they have been observed in large numbers within the same orchard. *Ulonemia leai* is the problematic species for the Atherton Tablelands region; though it also occurs in southern Queensland, it has not yet been reported from macadamias there. The last species, *U. concava*, was collected from large infestations near Maleny by Queensland DPI in the late 1960s. However, we have been unable to locate any specimens collected more recently than this, and the status of this species is unknown.

Population genetics

We used *U. decoris* as a model to determine the population structure of lace bugs across the Northern Rivers region. Our hypothesis was that lace bugs were restricted to how far they could migrate, so that the farther two populations of lace bugs were from each other, the less related they would be. We collected specimens from 7 different populations, with distances between populations at 300 m, 5 km, and 20 km. To determine relatedness, we extracted and sequenced portions of genomic DNA and examined these portions for variations.

Our results indicate that variation between populations of *U. decoris* is very low. This is counter to the prediction made in our hypothesis; there is likely a high degree of mobility of lace bugs between locations. The mechanism for their mobility is unknown, and could be natural (wind), artificial (carried by clothing/machinery), or both. However, it is clear that such high mobility enables lace bugs to re-colonise orchards where populations have been depleted due to pest management practices.

Conclusion

We hope that the information provided by the Macadamia Lace Bug Project will help inform pest management strategies for these insects. Several scientific publications from the project are nearing completion, and final results will be finalised in the coming months.

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