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SOME KEY QUESTIONS OF GROWERS (AND SCIENTISTS) ABOUT PESTS (apart from what chemical to use and when?)

- **Where do they come from into my grove? How do they sometimes just appear? And what can I do about it?**

- **How do they spread? How did they get into the state/district? How can we restrict their spread?**

- **What are their natural enemies and how can I encourage them?**

- **What are their hosts other than olives?**

- **What impact does tree health and nutrition have on these pests and their natural enemies?**

Olive lace bug (OLB), *Froggattia olivinia*, native species from eastern NSW and SE Qld which attacks plants in the Oleaceae.

Serious pest in eastern Australia; spread with movement of plant material, to Victoria, South Australia, Tasmania and particularly WA, where it is a serious pest.

Limited information about its natural enemies, although green lacewing (commercially produced) may have some effect. Lacewing release has been tried by a number of growers. Other lace bug species internationally have a number of natural enemies, most commonly egg parasites and predators.

Evidence that olive cultivars have different tolerances to OLB and that stressed trees are more attractive and more severely damaged than healthy trees.

While a number of registered insecticides including conventional and organic options are available for use against OLB in the field, the timing of applications for effective management is problematic, especially for growers without effective monitoring systems

Timing of applications and effective spray coverage are critical for success of most of these options, including organic options such as oil sprays and insecticidal soaps, which target early nymphal stages

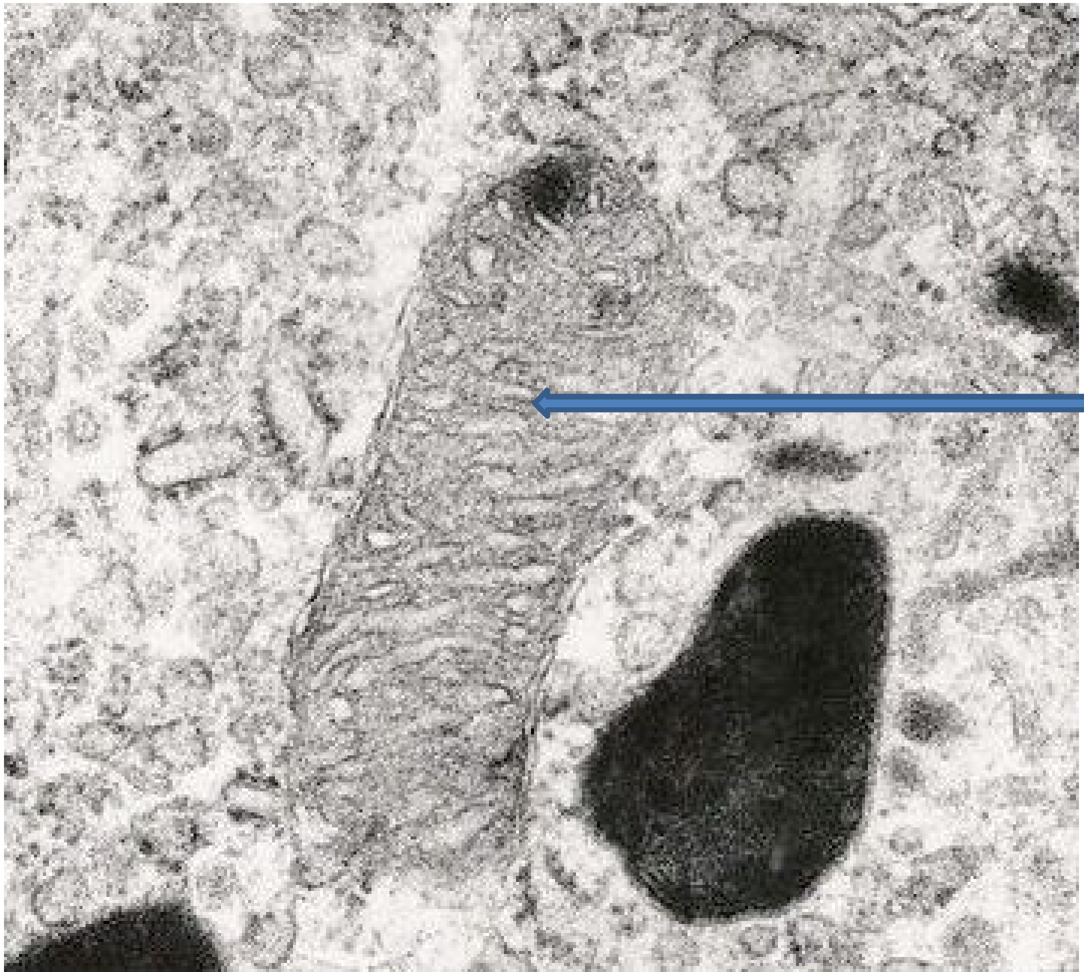
Others chemicals such as pyrethrum and synthetic pyrethroids are broad-spectrum and IPM-disruptive, but can be used effectively against adults or mixed populations

Non-chemical management options include restricting new infestations from nearby forests or groves, including on equipment, and trying to avoid stressing trees

Green lacewings may be of some use in suppressing populations

To answer a number of these questions posed, we have used DNA technology

We are using DNA from the cell mitochondria (mtDNA), the respiration energy powerhouse organelles



Mitochondrion in cell, showing cristae (Transmission Electron Microscopy)

mtDNA is more stable over time/conditions because:

- it is present in multiple copies and is located in a double membrane bound organelle
- It acts as a Lineage marker
- mtDNA is maternally inherited, as mitochondria are passed to offspring by females
- Some insects reproduce without fertilisation, so mtDNA is useful in sorting out lineage/relationships of populations.
- We used the mtDNA Cytochrome C oxidase subunit I (**CO1**), commonly used to determine lineage and relatedness of insect populations (i.e. their origins)

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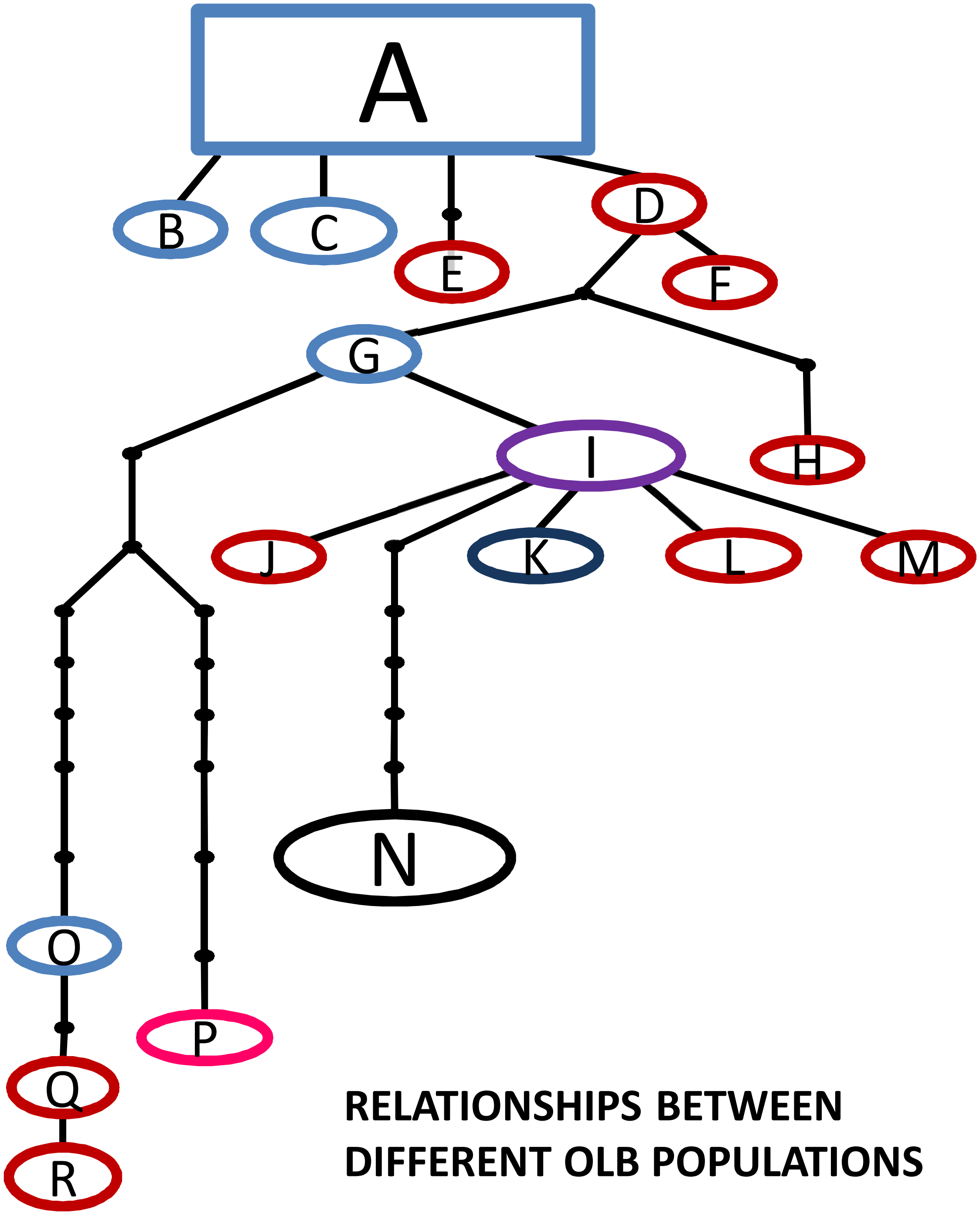
We used PCR (polymerised chain reaction) to amplify the mt-DNA , and the DNA products were sequenced from a number of OLB from each location. We compared DNA sequences to determine how similar they were within and between populations



RELATIONSHIPS BETWEEN DIFFERENT OLB POPULATIONS

Key to haplotype groups:

A: Hunter Valley, NSW (12)
Sydney Basin, NSW (6)
Mudgee, NSW (3),
Richmond, NSW, *O. europea*) (2)
Richmond, NSW, *Osmanthus* (3)
Canberra, ACT (1)
B: Sydney Basin (1)
C: Richmond, NSW, *N. longifolia* (3)
Richmond, NSW, *O. europea* (1)
D: SE QLD (1)
E: SE QLD (1)
F: SE QLD (1)
G: Sydney Basin, NSW (1)
Canberra, ACT (1)
H: SE QLD (1)
I: SE QLD (6)
Sydney Basin (1)
Richmond, NSW, *Osmanthus* (1)
J: SE QLD (1)
K: Boort, VIC (3)
L: SE QLD (3)
M: SE QLD (1)
N: SW WA (9)
Sydney Basin (2)
Canberra, ACT (1)
O: Sydney Basin (1)
P: Coonalpyn, SA (3)
Q: SE QLD (5)
R: SE QLD (1)

Our key initial findings indicate:

- a SA population is likely to have originated from NSW Sydney Basin/ Mudgee

- The movement and subsequent isolation of the SA population has led to some variation between the main group of NSW central coast/tablelands specimens (Group A) and the SA specimens

- In Group I the majority of specimens in this group are from SE QLD, but additional specimens from Sydney have the same genetic makeup

- The offshoot of Victorian specimens from this group, along with additional groups of SE QLD specimens point towards a likely origin of SE QLD

- WA populations also point towards a likely origin of SE QLD

- a notable amount of variation occurred between regions and locations, but some variation occurred between specimens from the same location

- No variation was found between specimens from the Hunter Valley, suggesting uniform populations

- More variation was recorded between specimens from locations in SE QLD than from NSW and Canberra (ACT), suggesting it is a centre of origin of OLB

OLB adults and nymphs on underside of olive leaf

The variation between specimens from the same region may be the result of the movement of plants between locations, including products of the nursery industry. The widespread presence of SE Qld types may also indicate spread around Australia via plant nurseries

The movement of olive harvesting and other equipment around locations may be another explanation for this variation

Following the results of this work, more OLB populations should be sampled and tested to gain a clearer picture

For future management of OLB, further research is needed to understand how infestations in olive groves occur, including how it spreads into previously unreported locations as well as into groves from native plant hosts in nearby vegetation

OLB nymphs hatching from eggs laid in underside of olive leaf

Olive black scale *Saisettia oleae* Cosmopolitan species, with wide host range, including citrus

Consistently regarded the most important and widespread olive pest in Australia

>24 species of beneficial insects, including 22 parasitoid wasp spp. released for black scale control, particularly in citrus

15 parasitoid species recorded in association with black scale in Australia.
Most common are wasp parasitoids *Scutellista caerulea* and *Metaphycus* spp.

Although natural enemies provide effective control of black scale in citrus, control in olives is often insufficient to maintain the pest below an economically damaging levels

Attempts made by Altman & Baker to mass rear and release 2 *Metaphycus* spp. in olive groves in WA, SA and Vic had limited success

***M. helvolus* (L)**
***M. bartletti* (R)**

Ants in association with black scale are a key factor negatively impacting biological control

Correctly timed oil sprays and insect growth regulators, e.g. pyriproxifen (Admiral ®) have been shown to be effective.

Key issues for implementing effective black scale management that need to be addressed are:

- understanding and predicting timing of generations for effective targeting of treatments
- optimizing scale natural enemies, especially parasitoids
- effective ant control, and
- Ensuring environmentally "soft" options and organically acceptable chemical treatments are correctly applied (timing and coverage).

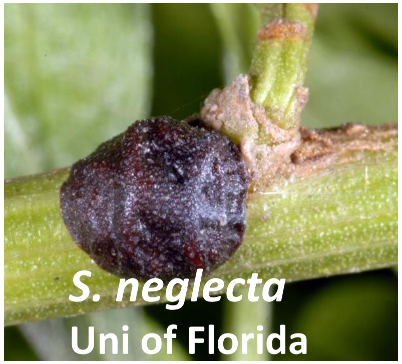
Ants attending black scale

Aims of PhD study at WSU (Phuong Sa)

- Black scale is a species within a complex of closely related taxa; Po et al. (2017) confirmed 3 species in Australia:

  Saissetia miranda Mexican black scale

  Saissetia neglecta Caribbean black scale



  Saissetia oleae Mediterranean/olive black scale

so: **determine whether *Saisettia oleae* is the only species present in temperate Australia on olives and citrus**

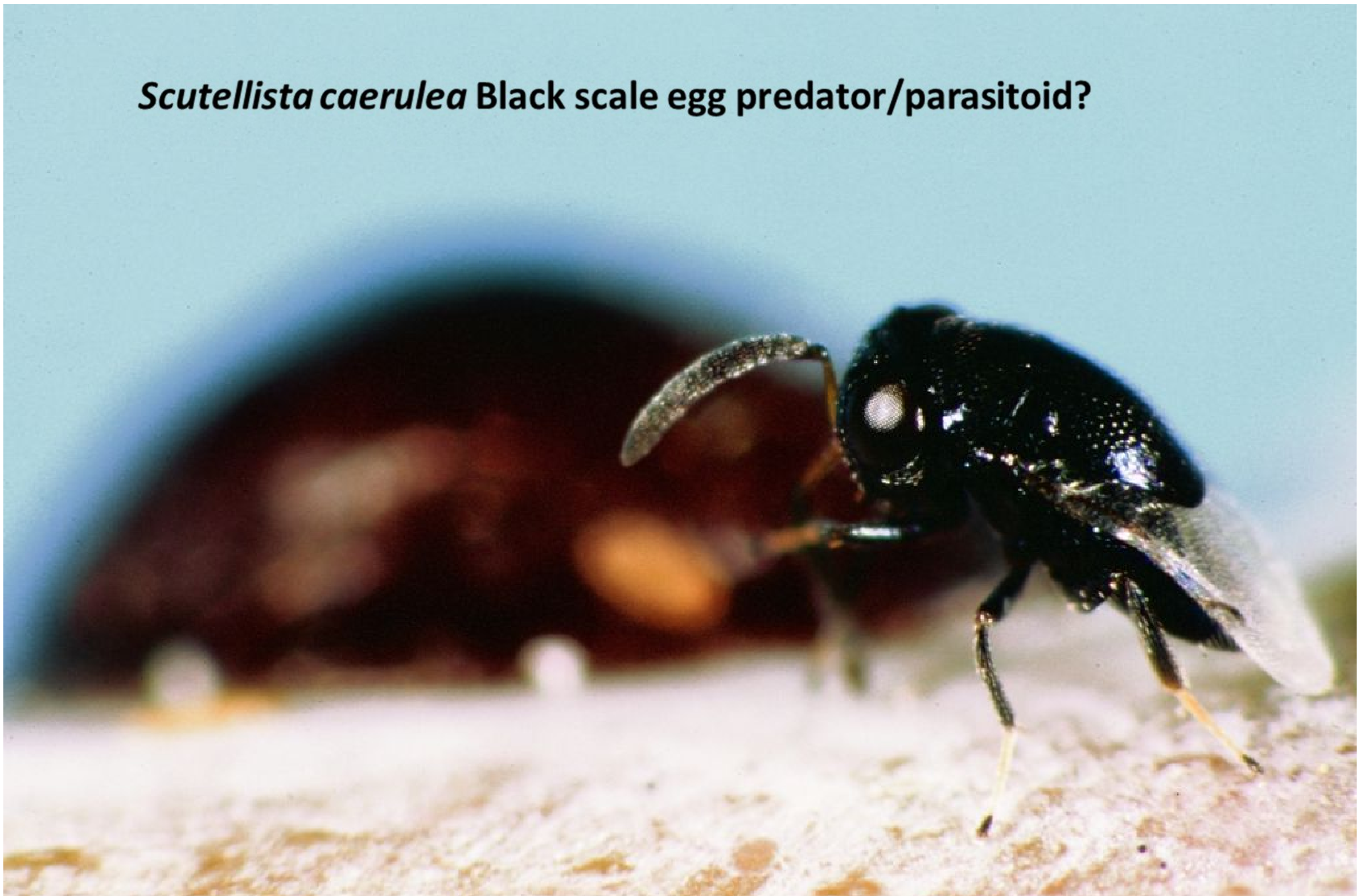
Debate exists regarding the number of larval instars of *Saisettia oleae*; so: **confirm the number of instars on olive and citrus**

Black scale has considerable variation in reported fecundity so: **assess the influence of host plants, including foliar nitrogen, on scale size and fecundity**

In addition to abiotic factors, such as foliar nitrogen, natural enemies affect the development of black scale so: **examine the impact of parasitoids and predators of *Saissetia oleae* in Australia**

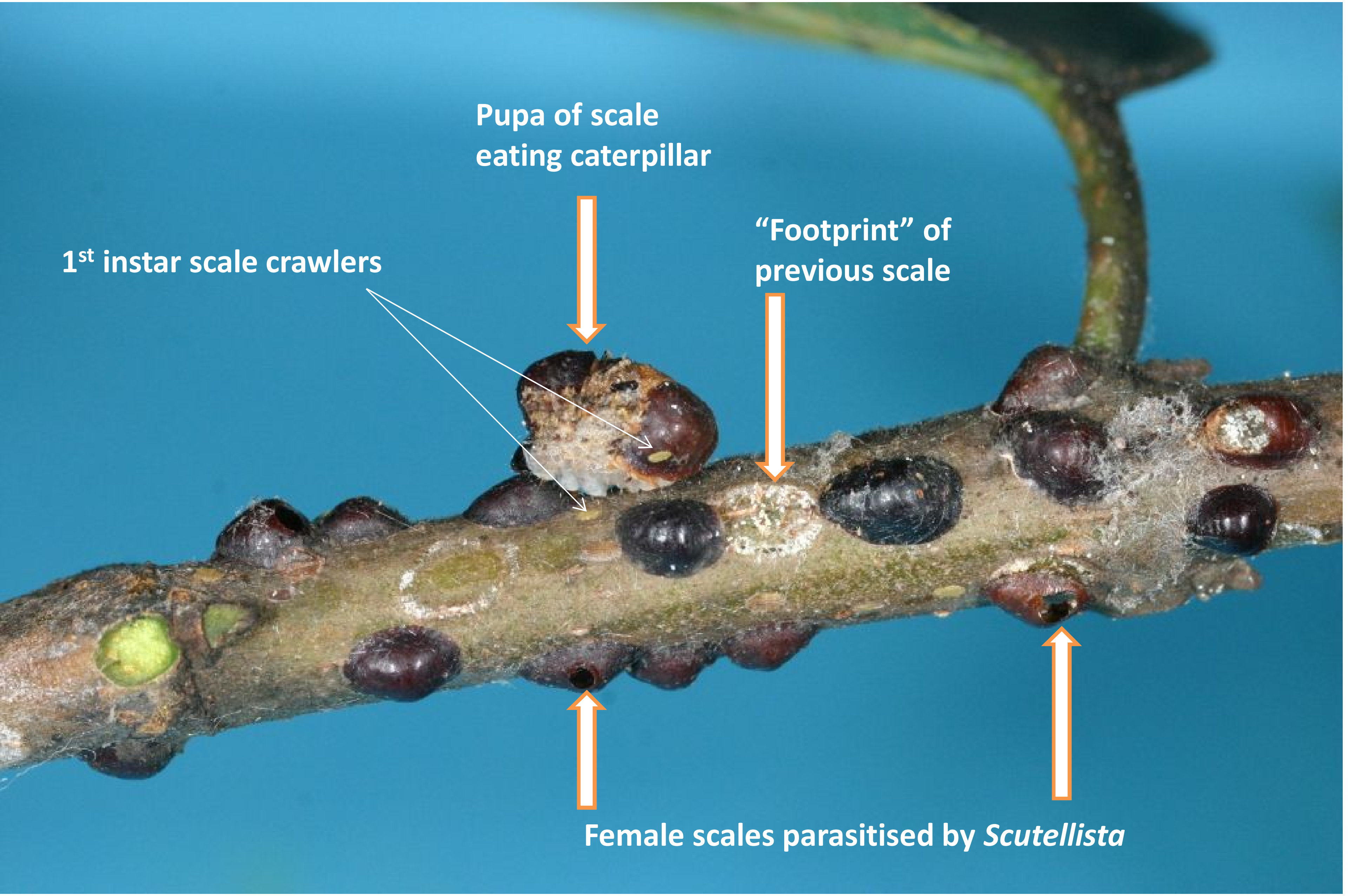
Female black scale lifted with eggs underneath

Host scale size may influence numbers of parasitoids/predators per host and their size on emergence so: **assess the relationships between rates of parasitism of black scale and hard wax scale by *Metaphycus* spp. and *Scutellista caerulea* in relation to host size**



Larvae of *Scutellista caerulea* have been reported to only feed on eggs of its host scale; however, other studies have reported they feed on body tissues adult female scales so: **determine whether larvae of *Scutellista caerulea* feed on non-egglaying host females**

Larva (grub) of *S. caerulea* among black scale eggs


Pupa of scale
eating caterpillar
1st instar scale crawlers
"Footprint" of
previous scale
Female scales parasitised by *Scutellista*

Black scale were collected from olives and citrus in temperate regions of Australia (NSW, Vic, SA, WA)

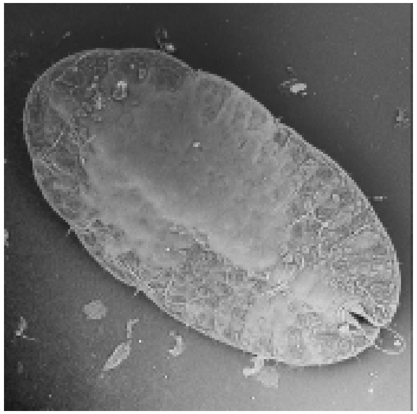
Scales were maintained in the laboratory until all parasitoids had emerged, and the number and species were determined

Identification of scales and parasitoids were undertaken using morphological (Electron microscopy) and genetic studies, using CO1 mt-DNA, to determine the species and any variation within and between populations

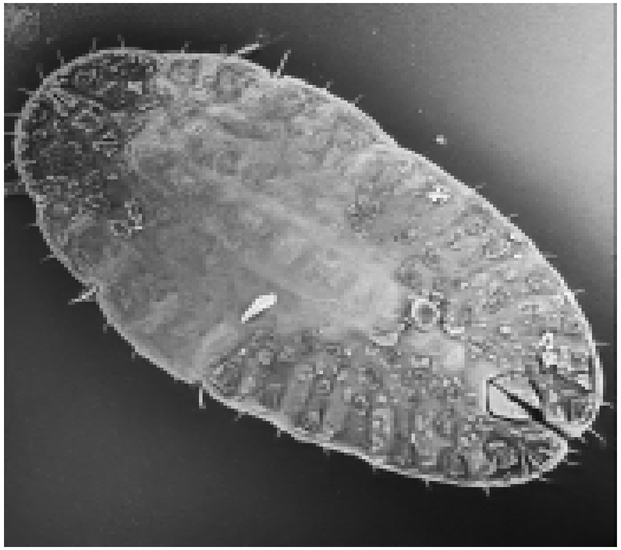
Young, immature female black scale

Results to date

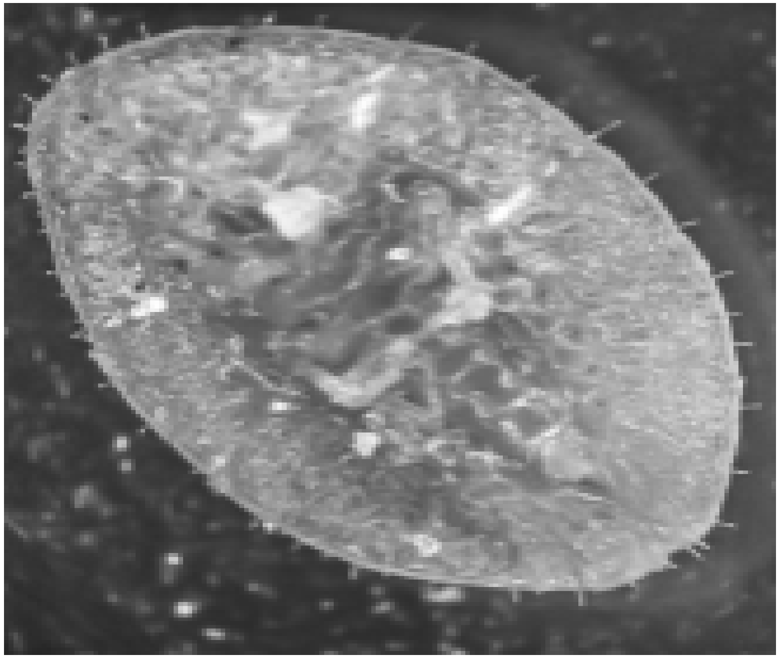
S. oleae has three instars on both olives and citrus that can be distinguished by the number of marginal setae (hairs/spines).



1st instar

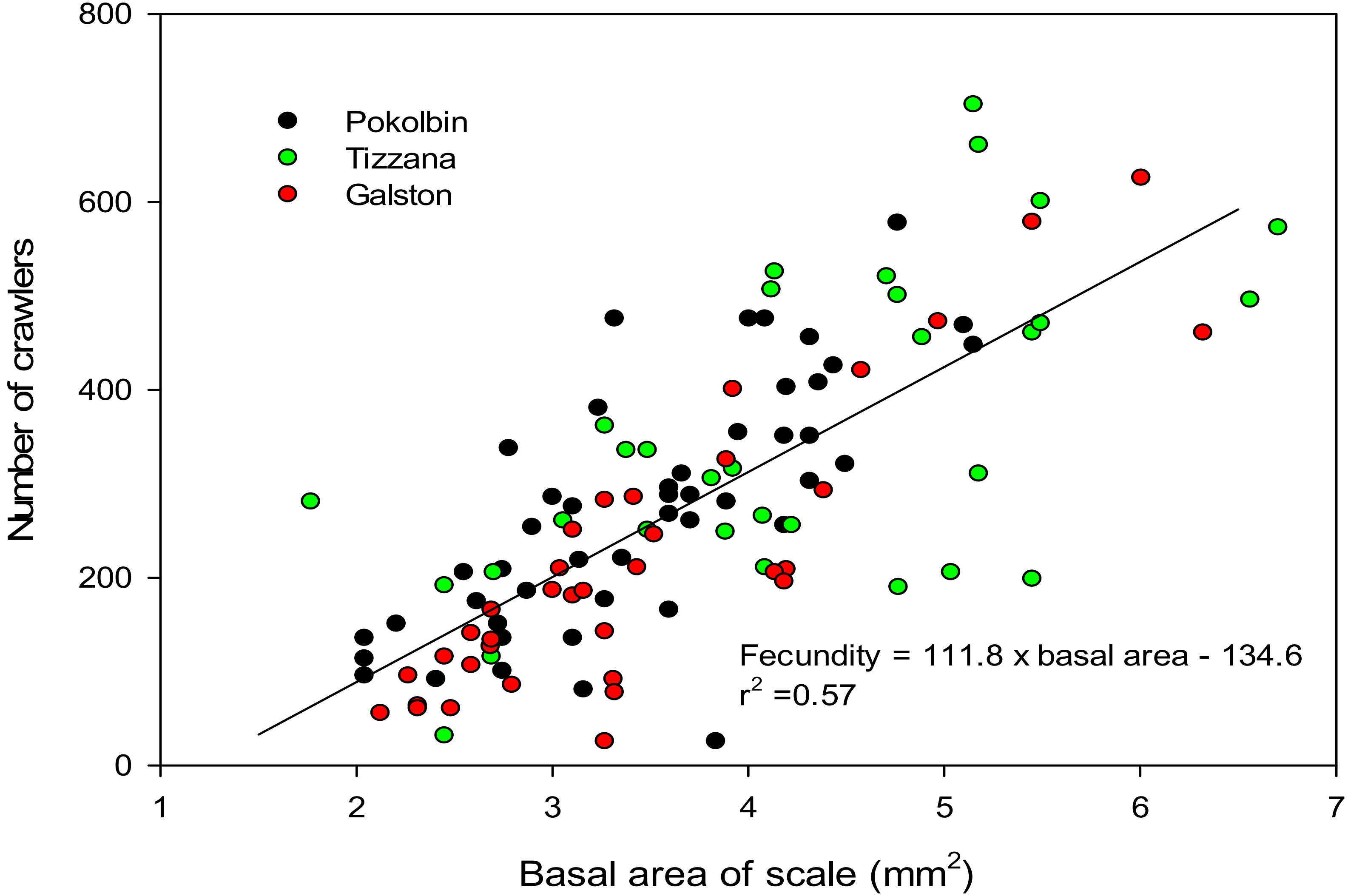


2nd instar



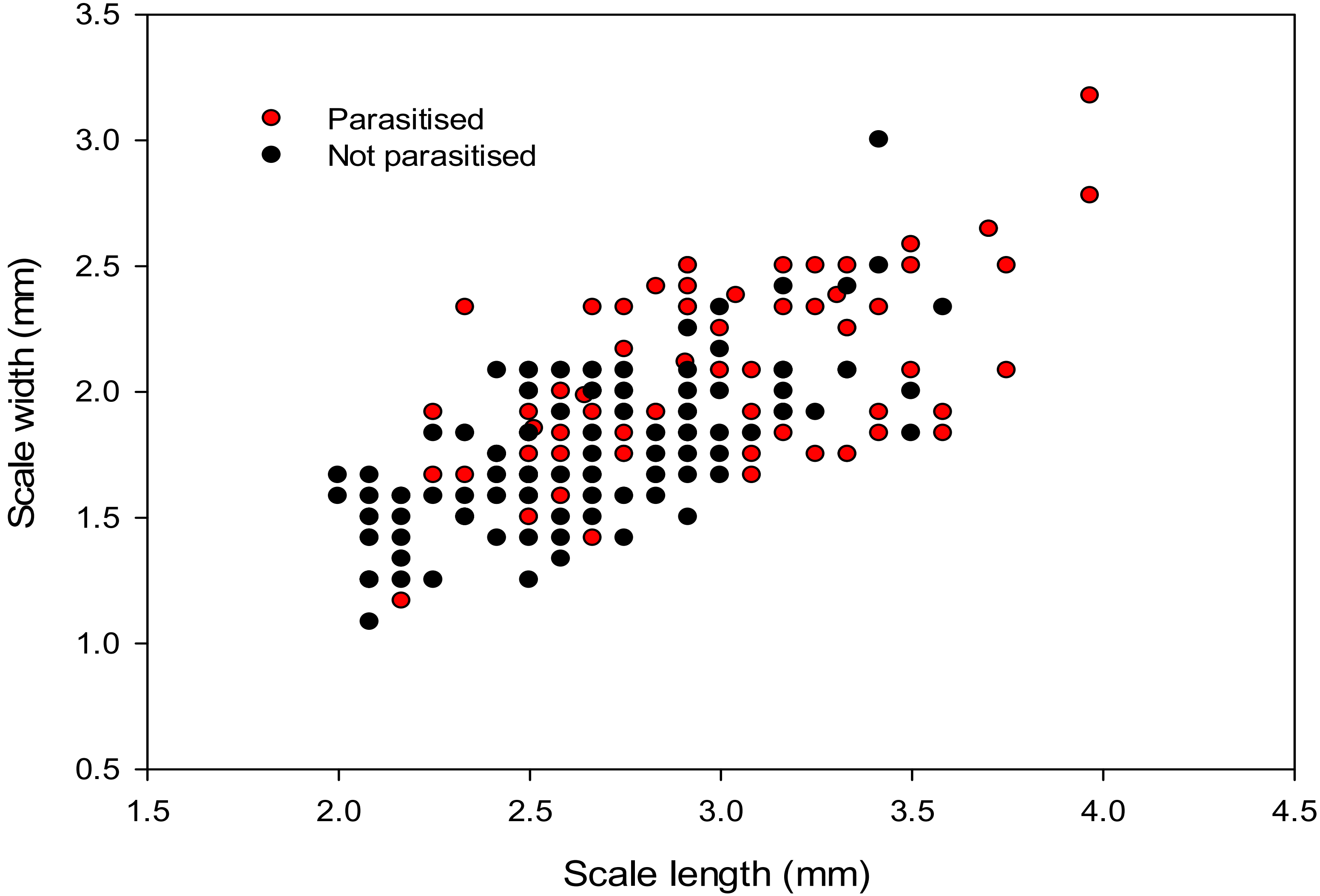
3rd instar

For non-parasitised black scale, as host size increased so did fecundity



Relationship between host size (area of basal surface of scale) and the number of crawlers produced by non-parasitised black scale at three locations.

Parasitised scales tended to be larger than non-parasitised scales



Relationship between dimensions of parasitised and non-parasitised black scale from a number of locations. Parasitised scales contained a single parasitoid.

Saisettia oleae was the only species of black scale found on olives

Increased plant nitrogen nutrition (and leaf nitrogen) increased size of scales

Parasitised scales produced on average 24 crawlers per individual and non-parasitised scales produced 277 crawlers

Scutellista caerulea fed on female scales, not just consumed eggs

Newly hatched nymphs emerging from under mature female black scale

SOME KEY TAKE HOME MESSAGES FROM THIS RECENT WORK

OLB can be spread to areas where they don't previously exist via movement of infested plants and equipment, including harvesting equipment and harvesting bins

OLB may come from surrounding forest areas where host plants in the family Oleaceae occur. The extent to which this happens in different districts isn't still clear

Parasitoids, especially *Scutellista caerulea* and *Metaphycus* spp. can play an important role in black scale management, by substantially reducing the number of emerging scale crawlers, if appropriately managed

Tree nutrition, especially nitrogen, can impact both black scale development and its parasitoid production

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