Preference and avoidance behaviour of Chrysomelid beetles using olfactory sensing of potential host-tree competitors

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Abstract

Chrysomelid beetles are a family of phytophagous insects that feed on nutritious young *Eucalyptus* leaves. Though they have been known to cause extensive damage to *Eucalyptus* crops, they are considered charismatic native insects in Australia. Comparatively, the steelblue sawfly (*Perga affinis),* more commonly known as 'spitfires' are known not only to affect, but strip entire trees of their leaves, particularly in Eucalyptus crops. In Kosciuszko National Park, NSW, we investigated the olfactory preferences of Chrysomelid beetles when presented with a variety of odours. We aimed to identify these beetles' attraction and avoidance behaviours to their potential competitor, the steelblue sawfly, using a Y-maze olfactometer. Results demonstrate a complex relationship between these herbivores and their host plant, and revealed Chrysomelid beetles' ability to sense competitors and resources, despite neither being visually accessible. Indeed, results indicate that these beetles use olfactory sensing to navigate their environment. Beetles were significantly more likely to choose leaves (food sources) over larvae of the competing species. These findings indicate that an increase in the *P. affinis* population in the area may cause the Chrysomelid beetles to be outcompeted. By understanding the preferences and avoidance behaviours of this beetle family, we can better manage and maintain their populations in the future.

Running title: Olfactory senses affecting host choice in defoliating *Eucalyptus* pests

Keywords: defoliating, Eucalyptus, herbivore, host choice, pests

Introduction

Leaf beetles (Coleoptera: Chrysomelidae) are a large family of phytophagous insects whose global economic significance has been extensively researched due to their detrimental effects on timber production (Duffy 2006; Nahrung *et al.* 2008; Henery *et al.* 2008; Müller & Müller 2017). Leaf beetles' ability to accelerate the death of host trees through defoliation as both adults and larvae positions them as a threat to forestry and the broader Australian environment. In Australia, Chrysomelid beetle-mediated mortality of *Eucalyptus* hosts has recently increased, particularly in large plantations (Nahrung *et al.* 2008; Henery *et al.* 2008; Magistrali *et al.* 2019). Although the larvae of these species are limited in movement, adults can disperse profusely by flying throughout the canopy of plantations to select the optimal tree (Tribe & Cillié 2000; Elek & Patel 2014).

Notably, the growth of pest Chrysomelid species, such as *Paropsis atomaria*, *Paropsisterna selmani* and *Trachymela sloani,* is limited by two main factors: foliage nutritional quality and temperature (Carne 1966; Henery, Henson *et al.* 2008; March *et al.* 2017). Should resources not allow them to accumulate sufficient body fat over winter, the adults will not survive to reproduce in the coming summer (Carne 1966; Marsh *et al.* 2017).

Stressed or damaged trees may not provide the nutrition necessary, and may even increase the cost of detoxification for insects (Henery, Wallis *et al.* 2008). Chrysomelid beetles prefer younger growth; adults will lay eggs on the end of a branch near the new growth and travel inwards along the branch for foraging on older leaves, where some feed on the margins of leaves (Carne 1962; Tribe & Cillié 2000; Reid & de Little 2013) while others clip off new shoots before they can even grow (Zhang *et al.* 2020). Larvae born from these eggs will feed gregariously on the substrate around them, remaining as a group (Carne 1962; Tribe & Cillié 2000).

In a similar manner to the Chrysomelid family, steelblue sawflies (*Perga affinis* Hymenoptera: Perigidae) are known to cause extensive damage to Eucalypt plantations (Neumann & Collett 1997). Known as 'primary' pests in forestry, these folivores target young succulent leaves at the top of the tree, on juvenile trees below 5 m. However, *P. affinis* has been to strip whole trees, or even entire stands, of their leaves (Neumann & Collett 1997). In their final instars between late winter and spring, *P. affinis* is most destructive, forming large clusters akin to those of the Chrysomelids (Carne 1962; Massaro & Cooper 2019). These larvae separate *Eucalyptus* oils from their food, sequestering their chemicals, which they may use to attack predators, thus earning the colloquial name 'spitfires' (Carne 1962). Similarly, larvae of Chrysomelid species utilise secondary metabolites to produce hydrogen cyanide, which they can release if threatened (Moore 1967).

Unlike some wood-boring insects, neither *P. affinis* nor members of the Chrysomelid family target stressed or damaged trees, preferring the higher nutritional quality of younger leaves to provide resources for their larvae. Although offspring are capable of moving metres across a tree to reach new shoots, their dispersal ability is limited to one tree until adulthood (Carne 1966; Nahrung *et al.* 2008). This presents a trade-off for both organisms. Optimal foraging theory states that adults should aim to maximise their performance by consuming the most abundant biomass (Stephens & Krebs 1986); however, optimal oviposition theory holds that adults should aim to oviposit on host plants that maximise the survival chances and fitness of their offspring (Jaenike 1978). Thus, the laying decisions of adult female Chrysomelids, Perigids and other folivorous insects on their host determine the survival of their offspring, which are limited in their dispersal ability (Carne *et al.* 1966; Carne *et al.* 1962; Neumann & Collett 1997; Nahrung *et al.* 2008; Tribe & Cillié 2000). The ability to determine the condition and quality of potential host trees is, therefore, important to the management of leaf-mining species (Henery *et al.* 2009), and predicts a level of competition between species of Chrysomelidsand Perigids when their distributions overlap.

When plants become damaged or stressed, they may release secondary metabolites – organic compounds that plants use to be competitive in their environment. These may act as attractants, induce flowering or induce deciduous behaviour (Teoh 2015). Volatile organic compounds (VOCs) are sensed as odours and may be released as an attractant or a warning. This can occur after damage from wood-boring or foliage-mining insects (Martínez *et al.* 2017), with the VOCs acting as attractants or deterrents to foliar herbivores (Matsuki *et al.* 2011). Moreover, these secondary metabolites allow specialist folivores to select the most favourable individuals within their host population (Matsuki *et al.* 2011). Insects use the sensing of VOCs to navigate their sensory world; thus, how one insect species experiences a particular VOC will be different from that of another species (Conchou *et al.* 2019).

Additionally, insects may also produce their own VOCs in the form of pheromones, used in mating and other communications. Odour receptors are parts of the brain that allow for the sensing of VOCs; they may differ in size and ability between and even within species (Andersson *et al.* 2015).

Recent studies have indicated that *Phoracanthus semipunctata*, a wood-boring beetle (Coleoptera: Cerambycidae) that is also a pest on *Eucalyptus* trees, may use olfactory cells to sense volatile semiochemicals produced by *Eucalyptus* species to select host trees. *Monochamus* species can co-opt pheromones produced by other bark-dwelling beetles to sense the condition of trees (Barata *et al.* 2000; Allison *et al.* 2001). The relationship between Chrysomelid *P. atomaria* and some plant volatiles has been investigated (Marsh *et al.* 2017), with findings indicating that secondary metabolite production in the form of a volatile group, known as oxidisable phenolic concentrations, does not influence the growth or survival of larvae of this species. Instead, leaf nitrogen content is thought to have a greater influence on *P. atomaria* host choice (Marsh *et al.* 2017). Similarly, Steinbauer (2010) found that although *Eucalyptus* species produce different oil concentrations at different latitudes, this did not appear to influence the distribution patterns of Chrysomelid beetles – nor did oxidisable phenolic concentrations.

As both Chrysomelids and Perigids occupy the same niche, further questions remain regarding the ability of one pest to identify the other and the effects this might have on potential host trees. Understanding the mechanisms underlying host-tree selection may benefit efforts to mitigate potentially detrimental population explosions of either the Chrysomelid beetles investigated in this study or *P. affinis*.

This study uses olfactometry to ascertain avoidance/preference behaviours of Chrysomelid beetles on host species *E. pauciflora* (in the alpine region of Australia) regarding the larval density of competitor sawflies. We hypothesised that, based on odour sensing, beetles would exhibit the following behaviour:

a) Move towards the leaves of host plants for sources of habitat and herbivory. Based on OOT, females may be more likely to choose host leaves than males.
b) Avoid sawfly larvae, because these present a competitive threat to Chrysomelid larvae. We expected females to show stronger avoidance than males due to competition for oviposition substrate.
c) Avoid sawfly-damaged leaves, because these would provide neither an optimal food source nor an optimal oviposition site. We expected females to demonstrate stronger deterrence than males, as this would provide neither valuable food nor an oviposition site.

Methods

This study was conducted in early December 2021 around Charlotte Pass in Kosciuszko National Park, NSW. We collected 77 Chrysomelid beetles from five sites between 1,630 m and 1,850 m in elevation. Each beetle was provided with a leaf from the site at which they were collected and separated into a single falcon tube with airholes and water available. Beetles were sexed under a dissection microscope following the directions of Maywald (2015), and identified to the genus or species level, where possible. Once sexed and identified, each beetle was measured for height, width and length, and given a unique identifying number.

A Y-maze is a tool commonly used for the assessment of spatial understanding and memory in rodents (Sarnyai *et al.* 2000; Kraeuter *et al.* 2019); it has also been used in assays for insect behaviour (Stelinski & Tiwari 2013; Camara Siqueira da Cunha *et al.* 2022). By attaching tubes to an air compressor, VOCs or other compounds that can be sensed can be

effectively pushed down the 'arms' to the 'foot' of the tube so that the Y-maze can be used as an olfactometer. This setup has been used to determine the effective use of oils as insect repellents in species of mosquito (Uniyal *et al.* 2016; Tyagi *et al.* 2017).

Four Y-mazes were set up following the diagram below (Figure 1). Each beetle was anaesthetised in a commercial freezer (-20°C) for two minutes to slow down movement when being placed in the sterile experimental environment from the individual's tube (Lee 1991). Beetles were placed at the bottom of the Y-maze. Each individual was given ten minutes to choose a target arm. A 'decision' was recorded if the beetle remained above a marking halfway up the 'arm' of the Y-maze, for 30 seconds or more. A decision was not recorded if the beetle travelled above halfway but remained for less than 30 seconds. If a decision was made, time taken was noted, and the beetle was removed from the Y-maze. If no decision was made, beetles were removed from the Y-maze after ten minutes had lapsed.
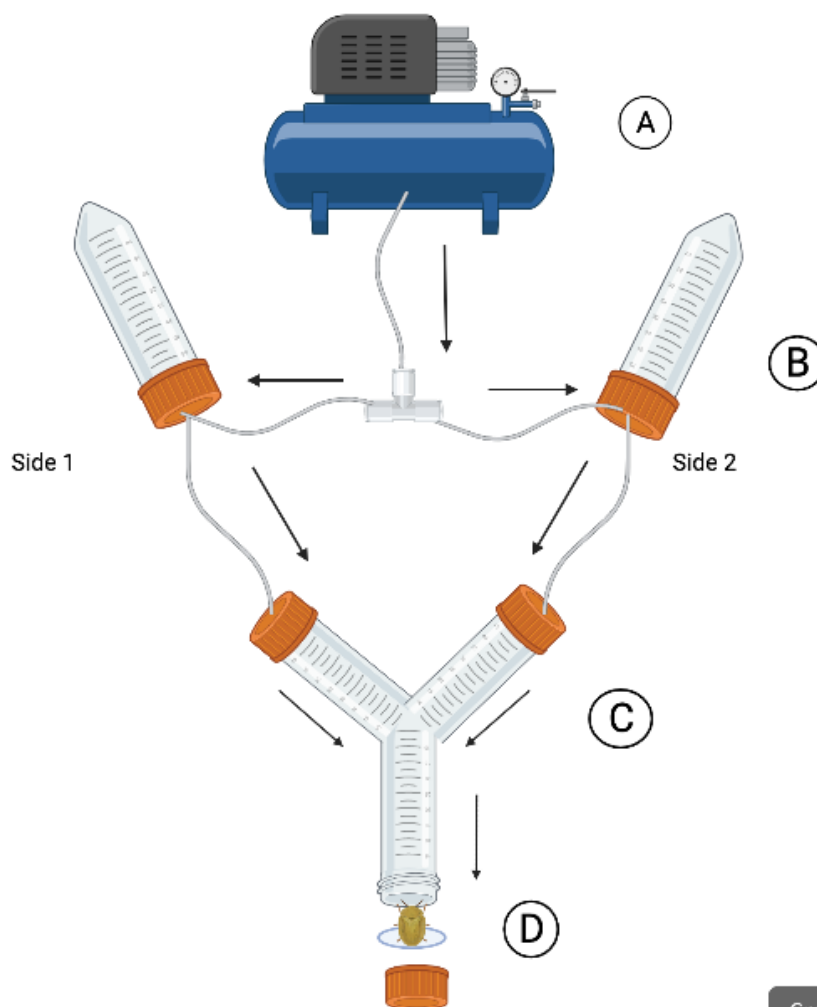
Figure 1: Laboratory setup for preference/avoidance experiments. A) Air is moved from the compressor to the odour tubes on Side 1 and Side 2. B) After circulating through the odour tube, the air is pushed through the connecting tubes to the Y-maze. Falcon tubes were glued with epoxy to create the 'Y' shape. C) Odour travels down one arm of the Y-maze towards the 'foot'. D) At the bottom, the beetle is inserted on a foam 'elevator', which is pushed up the maze until the beetle reaches the mid-point between the two arms.

A change to the method was made early, as beetles were not making decisions when placed at the 'foot' of the Y, after the first two controls (Table 1). Instead, a Styrofoam 'elevator' was measured for each Y-maze (Figure 1 (D)). Beetles were placed on the elevator after exposure to low temperatures and pushed up to the intersection of the Y to encourage

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decision-making. The treatments given to the beetles are detailed in Table 1. Leaves with 'mechanical damage' were subjected to three slices across the middle with a razor blade. Leaves with larval damage were taken from the enclosure containing coalescences of *P. affinis.*

Larval groupings of *P. affinis* were based on the research of Carne (1962), who found that larval coalescences could reach up to 30 individuals. To maintain an even number of larvae in each tube, 20 was used as the maximum (large) number of larvae. The minimum (small) number of larvae to maintain coalescence was five. Chrysomelid beetles were manually selected to balance genera and sex within and across treatments. Males were used as a control to measure female preference for different odours. In addition, efforts were made to prevent beetles from being frozen more than three times each, to prevent mortality. Treatments were repeated eight times on one side of the Y-maze, then swapped to the other side to adjust for directional biases. Overall, each treatment was replicated 16 times. No-decision (NA) results were recorded alongside decisions.

Table 1: Treatments in each side of Y-maze testing preference/avoidance of Chrysomelid beetles. Four controls were run before the four experiments were completed.

Side 1	Side 2
Controls	
Leaf (no damage)	Control (empty)
A small group of sawfly larvae (5 larvae)	Control (empty)
A large group of sawfly larvae (20 larvae)	Control (empty)
Leaf (sawfly larvae damage)	Leaf (mechanical damage)
Treatments	
Leaf (sawfly larvae damage)	Leaf (no damage)
A small group of sawfly larvae	A large group of sawfly larvae
A small group of sawfly larvae	Leaf (no damage)
3 undamaged leaves	1 undamaged leaf
A small group of larvae on leaf	Leaf (no damage)

Data analysis

All data analysis was completed using R Version 4.0.5 'Shake and Throw' (R Core Team 2021). Linear regression models, using the function *lm* in the library *dplyr* (Chambers & Hastie 1992) were used to determine the significance of preferences for each treatment. This function provides coefficients that indicate the significance of the relationship between treatments, and the value of the t-test. Box plots were created using *boxplot* in *dplyr*, to illustrate the differences in length of each genus (and the species within that genus, where possible) and any sexual dimorphism within genera. Additionally, bar graphs created using the library *ggplot2* were used to visualise the proportion of beetles that chose each treatment. Data collected from our experiments was binomial, and thus without a normal distribution (Lehmann *et al.* 2018). The effect of sex or genus on each treatment was determined using the same *lm* linear regression models from *dplyr* (Chambers & Hastie 1992).

Results

From the preference/avoidance data, we established the olfactory preferences of Chrysomelid beetles in the Y-maze olfactometer. During the identification process, we identified three genera of Chrysomelid beetles: *Paropsis*, *Paropsisterna* and *Trachymela*. Within these genera, the average length of the beetles varied greatly, with the *Paropsis* sp.

measuring considerably larger than other genera (Figure 2). Sexual dimorphism was also apparent within genera (Figure 3). The largest difference was recorded between males and females of the *Paropsis* genus.
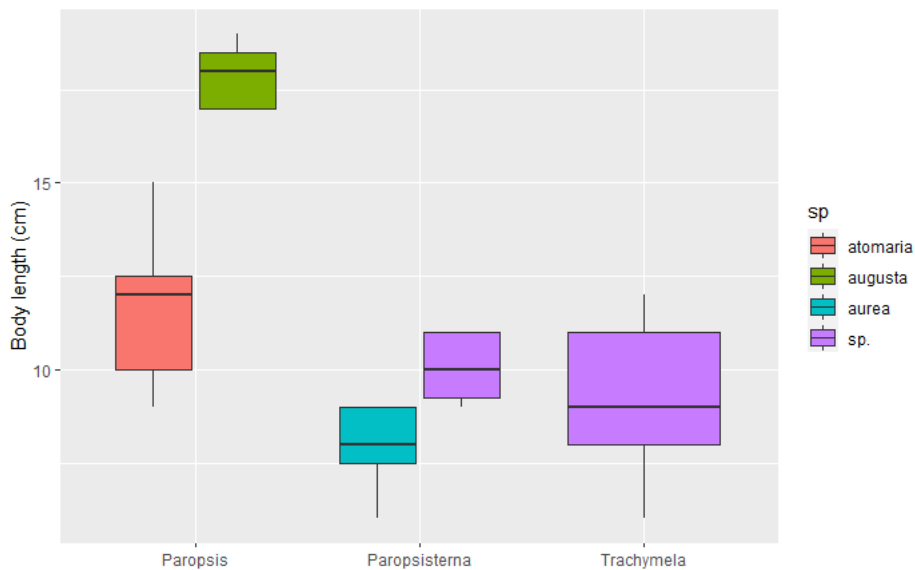

Figure 2: Body length of genera and associated species of Chrysomelid beetles used in Y-maze olfactometer assays. The mean is given as the centre line of each box plot. The species for each genus are indicated by different colours – individuals which were unable to be identified to the species level (annotated as sp.) are presented in purple.
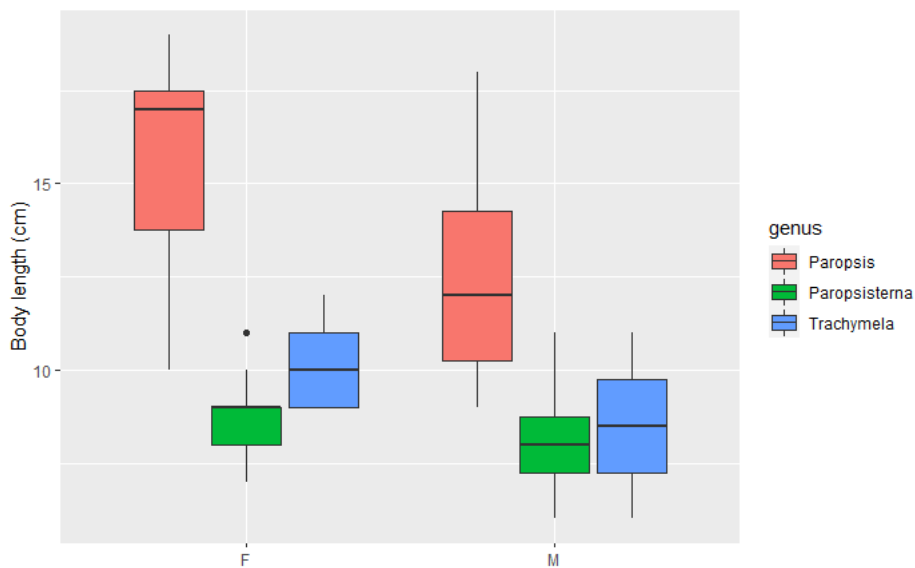
Figure 3: Box and Whisker Chart of sexual dimorphism for each genus of Chrysomelid beetle used in Y-maze olfactometer assays. The mean is given as the centre line in each plot. Females are notably larger than males within the Paropsis *genus, as well as within* Trachymela.

Controls

The controls revealed intriguing patterns of behaviour among the Chrysomelid beetles. Beetles significantly (p = 0.00824) preferred the leaf odour, although a small proportion chose the control over the odour source (Figure 4). Females were significantly more likely to choose the tube containing leaf odour than males. Comparatively, Chrysomelids were significantly (p = 0.0379) more likely to avoid small groups of larvae, preferring to walk

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towards the neutral odour source (empty/just air), or to make no decision at all (Figure 5). The third control somewhat contradicted this finding, indicating that a large group of larvae acted as an attractant to the beetles (Figure 6), although this relationship was insignificant. Beetles were equally likely to choose leaves damaged by *P. affinis* or via mechanical damage – however, our linear model indicated that male beetles were more likely to choose mechanical damage over larval damage (Figure 7). This significant result was overly dispersed and thus will be considered as a pattern rather than an indicative factor.

Treatments

Experiments were then used to investigate our hypotheses. Primarily, a leaf inflicted with damage from *P. affinis* larvae was compared to a leaf with no damage. Beetles preferred the leaf with larval damage when compared to a leaf with no damage, though not significantly (Figure 9). When a small group of larvae was compared to a leaf with no damage, considerably more beetles chose the leaf odour over that of the small group of larvae (Figure 8). This was contradicted by the next experiment, which found that more beetles preferred the odour of a large group of *P. affinis* larvae than a small group. Nonetheless, this result was not significant (Figure 11). The effect of leaf odour was tested using an experiment that compared three leaves of *E. pauciflora* with one. There was found to be no effect, as an equal number of beetles chose one leaf or three (Figure 12). Finally, we compared preferences for an undamaged *E. pauciflora* leaf with a leaf which was being eaten by five *P. affinis* larvae. The larvae were included in the tube of the Y-maze. A significant number of beetles preferred the undamaged leaf (p = 0.0474). Interestingly, *Paropsis* individuals were significantly more likely to make a decision than *Paropsisterna* individuals (p = 0.0474). No *Trachymela* species were included in this treatment
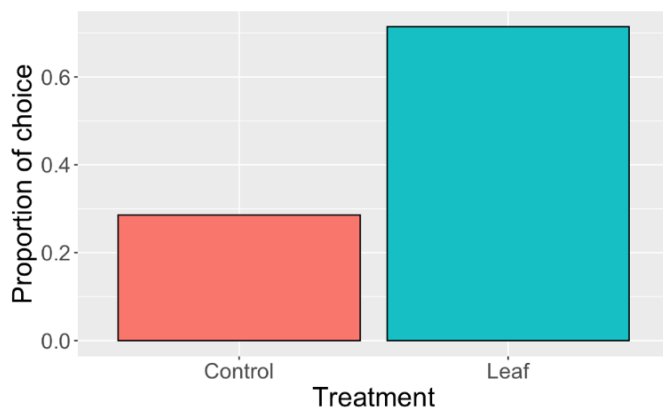
Controls

Figure 4: *Proportion of choice of Chrysomelid beetles for an undamaged leaf compared with no odour. A significant proportion of Chrysomelid beetles preferred leaf odour to the control (p = 0.00824).*
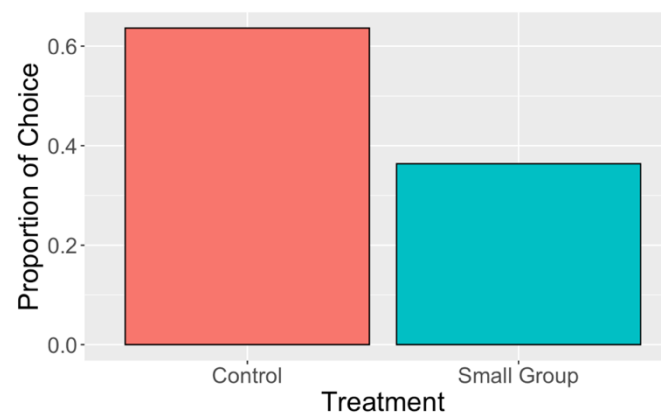

Figure 5: *Proportion of choice of Chrysomelid beetles between a small group of larvae compared to no odour. A significant proportion of beetles preferred the control odour over the small group of* P. affinis *larvae (p = 0.0379).*
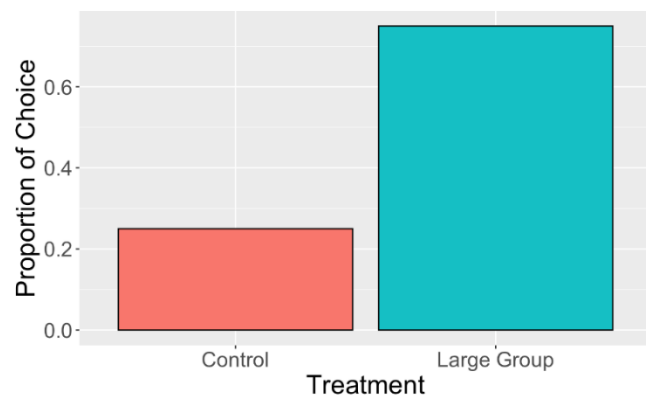

Figure 6*: Percentage of preference/avoidance of Chrysomelid beetles for a large group of larvae compared to no odour. A large proportion of beetles were attracted to the larval odour; however, this relationship was not significant (p = 0.293)*

Figure 7: *Proportion chart indicating Chrysomelid beetle preferences for leaves damaged by* P. affinis *larvae, or mechanical damage with a razor blade.*

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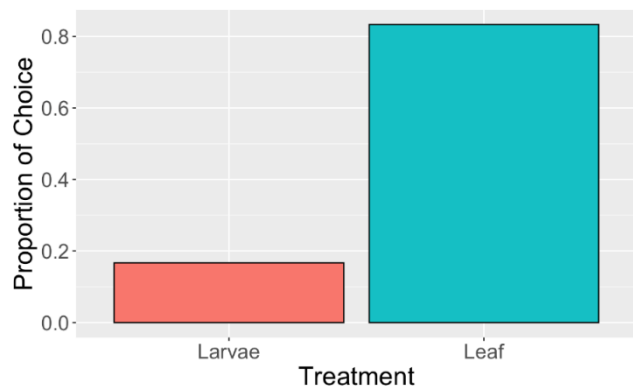
Treatments

Figure 8: *Proportion chart comparing Chrysomelid choice for the odour of a small group of* P. affinis *larvae with a leaf with no damage. Though considerably more beetles preferred the undamaged leaf over the small group of larvae, no significant relationship was found.*
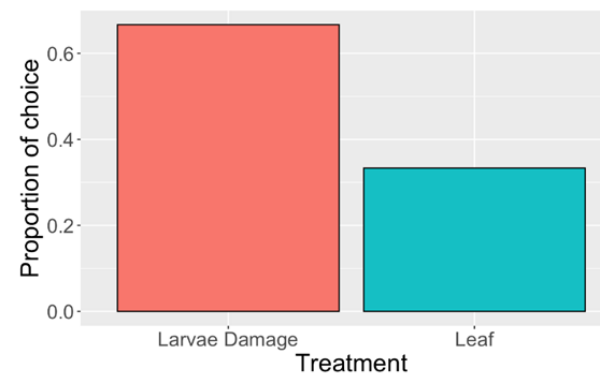

Figure 9: *Proportion chart comparing beetle preferences for a* Eucalyptus *leaf without* P. affinis *damage, to one with damage from the sawfly larvae. No significant relationship was found (p = 0.339).*
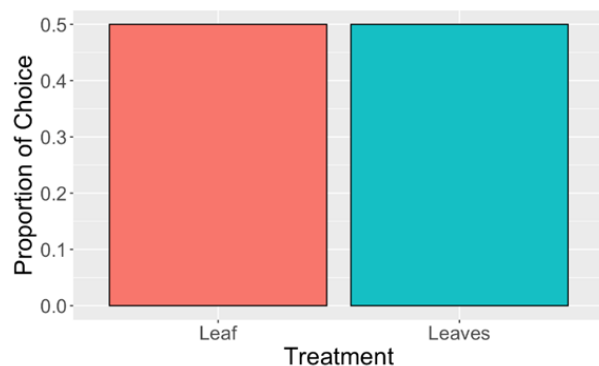

Figure 10: *Proportion chart comparing the preferences of Chrysomelid beetles for the odour of three leaves, or one leaf, from the same tree (*E. pauciflora*). Leaves were undamaged, and left in the tube for the same amount of time. No significant relationship was found (p = 0.161).*

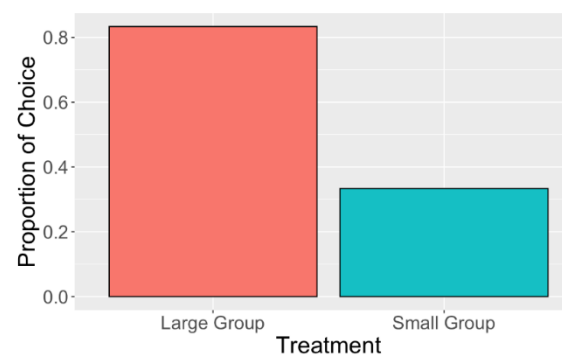
Figure 11: *Proportion chart comparing the preferences of Chrysomelid beetles for the odour of a large group of larvae (n = 20) with a small group (n = 5). No significant relationship was found, although more beetles preferred the large over the small group.*
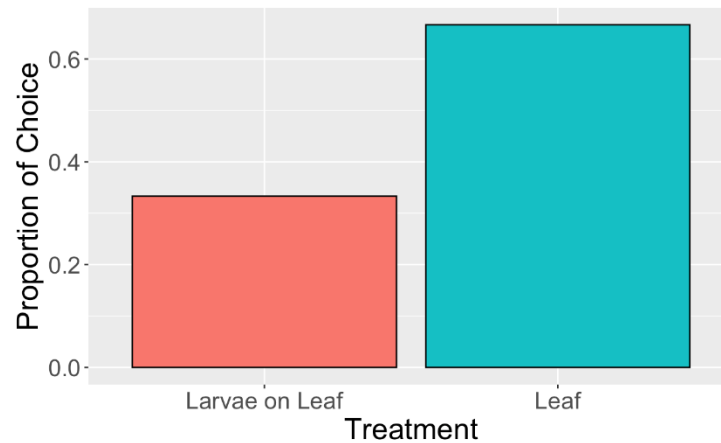
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Figure 12: *Proportion chart demonstrating Chrysomelid beetle preferences for an undamaged leaf when compared to five larvae on a leaf. Both leaves were from the same tree (*E. pauciflora*). A significant difference was found between beetle preference (p = 0.0474).*

Decisions were only made in 40% of all 148 trials (n = 60). Significantly more trials ended with no decision being made. Further data analysis was necessary to determine the proportion of decision-making beetles out of those tested, and whether this was determined by sex or genus. Although anecdotally, it seemed that those of the *Paropsis* genus were more likely to make a decision; the use of the general linear model determined no effects of sex or genus on the outcomes of decision-making.

Discussion

Decision-making

Of the 148 trials, in only 60 was a decision reached, while in 88 trials, the ten-minute time limit was reached with no decision. Females were significantly more likely to decide than males (p = 0.0409). Through observation, the genus *Paropsis* seemed more likely to decide than other genera. This may have been due to their size, as smaller beetles may have taken longer to recover from the freezing method. This conforms to our hypothesis that females would be more likely to make decisions due to optimal oviposition and foraging theories – however, this goes against our hypothesis in the instance where the decision made was to move towards the larvae. This difference may be linked to sexual dimorphism in odour receptors: females may have more developed odour receptors for sensing male sex pheromones in the wild (Hildebrand 1995; Rössler *et al.* 1998). Additionally, when considering optimal oviposition theory, females may need to be generally decisive when it comes to substrate choice and competition, to protect potential offspring (Jaenike 1978)

Odour sensing of leaves

Consistent with our first hypothesis, beetles preferentially moved towards leaves when compared to a control. Insects use odour receptors to find hosts, food and mates – indeed, any interaction with these factors is strongly regulated by the volatiles that an insect encounters (Martin *et al.* 2011). Thus, our results confirmed that Chrysomelid beetles can sense the odour from *E. pauciflora* and preferentially move towards it (Figure 4). In addition, it confirmed that our methodology functioned as intended, albeit with some alterations.

No effect was found of sex or genus on this preference – rather, the results were found to be on a population level. Conversely, no difference was found between beetles deciding to travel towards the odour of multiple leaves over one leaf, implying that either odour sensing of Chrysomelid

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beetles cannot discern between large and small amounts of leaves, or that beetles cannot discern between these leaf quantities (Figure 7). When comparing damaged and undamaged leaves, beetles appeared to have no significant preference (Figure 9). This demonstrates that the volatiles produced by the leaves may be perceived by the beetle similarly, despite damage on one.

These findings indicate that although volatiles from a specific ecological factor (such as a food resource) may be received by specific ORs in the insect's brain (Andersson *et al.* 2015), the insect may not perceive the quantity of this volatile. Interestingly, this does not reflect the findings of Lhomme *et al.* (2018), who found that the moth *Spodoptera littoralis* is capable of determining food quality with olfactory sensing, or Elliot *et al.* (2001), who found the ladybird *Hippodamia convergens* to be significantly more attracted to aphid-infested plants. Potentially, this could indicate that Chrysomelid beetles do not determine leaves with 'damage' to be of lesser quality than those that are undamaged, or that the damage done to the leaves did not release VOCs that were more attractive to the beetles. Further studies should investigate whether a larger quantity of leaves elicits a faster decision than those in the scope of this study.

Odour sensing of larvae

We hypothesised that Chrysomelid beetles would be deterred by sawfly larvae. Small groups of *P. affinis* larvae acted as deterrents to Chrysomelid beetles, but larger groups acted as attractants (Figures 5 & 6). A significant proportion of beetles were found to prefer the control odour over the small group of larvae (p = 0.0379). These findings align with our second hypothesis, that Chrysomelid beetles should avoid their sawfly larvae competitors. Like most insects, larvae produce pheromones to communicate with conspecifics (Fleischer & Krieger 2018); these pheromones may also be sensed by Chrysomelid beetles, and act as a deterrent. This was confirmed by both our fifth and final treatments (Figures 8 & 12). Beetles significantly preferentially moved towards the undamaged leaf in both experiments, rather than a small group of larvae on their own, or larvae on an undamaged leaf. Primarily, these findings confirmed that Chrysomelid beetles use olfactory senses to discern between the leaves of the potential host and the presence of competitors on the potential host through pheromones. Beetles were significantly more likely to move towards the odour of the host plant, indicating that they can sense other species through pheromones, or other compounds. Further, this gives strength to our hypothesis that sawflies may constitute a competitor for Chrysomelid beetles where their ranges overlap.

Contrary to the above, results from subsequent assays demonstrated the opposite effect. When compared to no odour, beetles preferred the odour of a large (n = 20) group of *P. affinis* larvae (Figure 6). Additionally, when a small group of larvae was compared to a large group, beetles preferentially decided on the arm closest to the large group (Figure 11). Notably, these results were not significant – however, the pattern is worth describing. Although pheromones may act as deterrents, they may also be released to communicate with other conspecifics (Noël *et al.* 2023). Large groups of larvae may not just act as a physical, visual indicator but may release these pheromones for conspecifics, which can then be 'hijacked' by Chrysomelid beetles to locate the food source (Fleischer & Krieger 2018).

Considerable offspring mortality has been recorded in the well-studied Chrysomelid species *Paropsis atomaria*, when larvae are fed trees found to be resistant to their feeding (Henery *et al.* 2008). These trees were not just of different species; rather, they were trees of the same species with differing resistance to insect herbivory. This indicates that the production of secondary metabolites is highly varied even between trees of the same species, and further suggests that sawfly offspring could be used as an indicator of susceptible trees; hence, a large coalescence of larvae may indicate a tree appropriate for oviposition and adult foraging by Chrysomelid beetles.

Beetles did not make decisions based on differences between leaves that were damaged by a razor blade (mechanical damage) and those caused by *P. affinis* larvae (Figure 7). This indicates that *Eucalyptus* species may produce general secondary metabolites when damaged rather than

compounds specific to herbivory and other kinds of damage. Alternatively, this may indicate that the olfactory sensing of Chrysomelid beetles is not sensitive to changes in semiochemical regimes – any output of volatiles from a *Eucalyptus* plant could be enough to elicit a response from the beetle. A study conducted by Acevedo *et al.* (2015) indicated that the lack of change in the chemical responses of the tree could be due to the specific plant–herbivore relationship – that chewing of *P. affinis* larvae is somewhat 'tolerated' by the tree, and does not affect the volatiles produced. In line with our earlier findings, chewing by larvae could indicate optimal foraging territory for Chrysomelid beetles.

Limitations

Factors within the experimental setup may have influenced the results produced by this experiment. Only one overhead light pre-existed in the lab, and though we maintained the position of the Y-mazes underneath, some were closer to the light than others. Light and light pollution are known to attract insects of many varieties (Justice & Justice 2016); thus, having the central light may have skewed results, drawing insects away from the darker sides of the room. Some Y-mazes were made with large falcon tubes (50 ml) and others with 20 ml tubes. Although only small beetles were used in the smaller tubes, the confined space may have affected the potency of the odour and affected the decision-making of individual beetles.

The freezing of beetles was conducted to anaesthetise them, reducing the stress of being handled and moved into a new environment (Attygalle *et al.* 2020); however, this also may have caused a delay or impairment in beetles' decision-making extending beyond the allocated ten minutes per beetle. Because no comparative tests were conducted on non-frozen beetles, further studies should address this factor in their methodology to reduce the potential impact of this limitation.

This experiment was not blinded – the person watching for decisions recorded the beetle identification number. One person recorded each decision, who also counted the time over the decision line. A double-blinded experiment could have prevented human error. Finally, there were considerably more *Trachymela* and *Paropsisterna* trapped than *Paropsis,* due to variation in accessibility across the landscape. Beetles of the *Paropsis* genus were needed for more replicates than individuals of other genera, and thus may have experienced some learning (Xue *et al.* 2007; Soliman & Hay 1978). Future research could include beetle learning as a random effect, or exclude individual beetles that have been used in one trial. Further, field observations could be useful in determining the effects of *P. affinis* on Chrysomelid beetle populations throughout their lifecycle, and over time.

Conclusions

Our results indicate that Chrysomelid beetles in the alpine region of Kosciuszko National Park use olfactory sensing to identify potential competitors and food sources. Our findings suggest an effect of sex, but not genus, on odour preferencing. Although there was a balance of male and female beetles in the population and those used for trials, females were found to be more likely to be decisive than males. In accordance with the optimal oviposition and optimal foraging theories, this supports our hypothesis that females are more likely to be decisive. However, although beetles avoided larvae in small groups, they moved towards larger groups of larvae. We theorise that a large group of larvae indicates optimal foraging territory, although further research is needed to test which specific compound can be sensed by the Chrysomelid beetles.

Finally, our results act as preliminary indications that the compounds produced by *Eucalyptus* leaves when damaged by herbivory are general (rather than specialised) secondary metabolites and the same as VOCs produced by mechanical damage from breakage. However, without specific measurement of these compounds, it is outside the scope of this study to make conclusions on this matter. Further research is needed to properly understand the differences between these compounds.

Because both the Chrysomelid beetle family and the sawfly *P. affinis* are known pests of *Eucalyptus* species both in Australia and internationally, understanding these host-seeking mechanisms will facilitate management and maintenance of these trees in a changing climate, while mitigating the impact of this family and their competitors on plantations of *Eucalyptus* species. These olfactometry assays should be used as a stepping stone to assays and observations in the wild as well as common garden experiments to better understand and predict these species' effects on their changing environment.

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