

## ORIGINAL CONTRIBUTION

**Role of olfaction in host plant selection and local adaptation of a polyphagous herbivore, *Eucolaspis* Sharp**P. R. C. Doddala<sup>1,\*</sup>, M. A. Minor<sup>1,a</sup>, Q. Wang<sup>1</sup>, D. J. Rogers<sup>2</sup>, E. M. Koot<sup>1</sup> & S. A. Trewick<sup>1,a</sup><sup>1</sup> Institute of Agriculture and Environment, Massey University, Palmerston North, New Zealand<sup>2</sup> The New Zealand Institute for Plant and Food Research Limited, Havelock North, New Zealand**Keywords**

attraction, bronze beetle, feeding preference, host plant volatiles

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<sup>a</sup>Contributed equally.**Abstract**

Host plant cues are known to shape insect–host plant association in many insect groups. More pronounced associations are generally manifested in specialist herbivores, but little is known in generalist herbivores. We used a polyphagous native beetle from New Zealand, bronze beetle, *Eucolaspis* sp. ‘Hawkes Bay’ (Chrysomelidae: Eumolpinae) to explore the role of olfaction in locating host plants and local adaptation. We also tested the role of other cues in the degree of acceptance or rejection of hosts. Adult *Eucolaspis* beetles were attracted to fresh leaf volatiles from apple and blackberry (Rosaceae). Male and female beetles responded similarly to olfactory cues of host plants. An indication of evolutionary affiliation was observed in olfactory preferences of geographically isolated conspecific populations. We found that geographically isolated populations of the beetles differ in their olfactory responses and exhibit some degree of local adaptation. However, irrespective of geographical and ecological associations, blackberry was preferred over apple as a feeding plant, and another novel plant, bush lawyer (*Rubus australis*), was readily accepted by 53.25% of the tested beetles. We show that plant volatiles play an important role in host location by *Eucolaspis*, but the acceptance or rejection of a particular host could also involve visual and contact cues.

**Introduction**

*Eucolaspis*, a native Eumolpinae (Coleoptera: Chrysomelidae) genus of New Zealand, comprises polyphagous species commonly called as ‘bronze beetles’. Recent findings on morphology, distribution and phylogeny of *Eucolaspis* suggest fewer endemic species (~4) than formerly described (~15) (Doddala 2012). *Eucolaspis* beetles were first reported on flowers of a New Zealand native shrub, manuka (*Leptospermum scoparium*, Myrtaceae) (Fabricius 1781 in White 1846). The beetles of the genus have since been recorded feeding on at least 67 other plant species in New Zealand, including 27 natives and 40 exotics (Table S1). Among the 33 plant families represented, the most common hosts are Myrtaceae and Rosaceae (11 and 6 species, respectively), but host plants range from small pasture herbs (e.g. *Trifolium*) and terrestrial orchids

(e.g. *Cymbidium*) to conifers (e.g. *Pinus*). It appears that the beetles have a particular preference for exotic fruit plants of the family Rosaceae, and orchards often support large populations resulting in significant economic damage. Fruit crops from families other than Rosaceae such as avocado (Lauraceae), black currant and gooseberry (Grossulariaceae), blueberry (Ericaceae) and feijoa (Myrtaceae) are also damaged. Early records suggest that host-range expansion in *Eucolaspis* may have occurred soon after European colonization and introduction of fruit crops in New Zealand (Huntley 1867).

Infestation of host plants by different species of *Eucolaspis* depends on local and regional distribution, as revealed from host plant use by different genetic lineages in New Zealand (Doddala 2012). We also suspect some level of intraspecific variation in host plant use by bronze beetles, as seen in other leaf beetles

such as *Chrysomela lapponica* (Coleoptera: Chrysomelidae) (Zvereva et al. 2010) and *Agelasa nigriceps* (Coleoptera: Chrysomelidae) (Kohyama et al. 2012). We are particularly interested in the *Eucolaspis* sp. 'Hawkes Bay' lineage, which infests apple trees and also occurs on exotic blackberry, linden, white clover, broad-leaved dock, native manuka and totara trees and possibly other plant species (Doddala 2012).

Long-term association with a host plant might lead to stronger preference, through tolerance, detoxification and recognition, which can have important implications for infestation of crop species and other hosts (Jermy 1984; Mostafa et al. 2011). Some geographically isolated conspecific populations of *Eucolaspis* sp. 'Hawkes Bay' persist on different host plants (apple and blackberry), suggesting local adaptation. It is not known whether these allopatric conspecific populations exhibit differences in sensory abilities in locating and selecting respective host plants. Any such differences could impact pest control methods such as behavioural control exploiting host plant stimuli. Hence, from both economic and scientific perspectives, it is important to assess intraspecific differences in host plant choice and location in *Eucolaspis* sp. 'Hawkes Bay' beetles leading to local adaptation.

In Chrysomelidae, cues that mediate attraction to host plants include plant size (Colorado potato beetle *Leptinotarsa decemlineata* – (Hoy et al. 2000)), other visual cues (e.g. *L. decemlineata* – (Szentesi et al. 2002) and *Phyllotreta striolata* – (Yang et al. 2003)) and plant volatiles (e.g. *L. decemlineata* – (Bolter et al. 1997; Schütz et al. 1997) and the strawberry leaf beetle, *Galerucella vittaticollis* – (Hori et al. 2006)). Among different host-finding cues, olfactory cues are the most frequently used (Stenberg and Ericson 2007). At least twelve leaf beetle genera show attraction to host plant volatiles (Fernandez and Hilker 2007; Stenberg and Ericson 2007; El-Sayed 2014). None of the previously studied genera belongs to the subfamily Eumolpinae, which remains poorly studied in this respect.

We examined preference for host plants among different populations of bronze beetles and assessed the extent of preference as determined by evolutionary association. The specific objectives of this research were to evaluate whether the bronze beetles show attraction to host plant odours and to explore whether allopatric conspecific populations vary in sensitivity to olfactory, gustatory and other contact cues in host plant preference. We conducted olfactory and feeding bioassays involving various known host plants and a novel plant, with two conspecific populations of *Eucolaspis* sp. 'Hawkes Bay' adult beetles.

## Materials and Methods

Normal seemingly healthy (showed no impairment in walking, flying and feeding) adult beetles were used for all bioassays. The test beetles originated from two different populations – an 'apple population' from an organic apple orchard ('Royal Gala') in Havelock North (Hawkes Bay, New Zealand) and a 'blackberry population' from a blackberry scrub in Waikanae (Kapiti Coast, New Zealand). The two populations used will be referred as 'Havelock North apple' and 'Waikanae blackberry' populations from this point forward. Conspecificity of these two populations was confirmed through the analysis of mitochondrial DNA and male genitalia (Doddala 2012). Insect collection, rearing and maintenance have been followed as previously described (Doddala 2012). Beetles used in the study were starved for 4 h prior to bioassays. Starving is necessary to induce host finding or feeding and is a widely used practice in similar experiments with herbivorous insects (García-Robledo and Horvitz 2009).

The role of olfaction in host detection was tested initially with Havelock North apple population beetles in Y-tube bioassays (no-choice: host plant vs. clean air) using four plant species: apple (*Malus domestica* CV 'Royal Gala', Rosaceae), blackberry (*Rubus fruticosus*, Rosaceae), white clover (*Trifolium repens*, Fabaceae) and broad-leaved dock (*Rumex obtusifolius*, Polygonaceae). White clover and broad-leaved dock are predominant under-storey plants in organic apple orchards in New Zealand, and both these plant species are infested by bronze beetles in the field (P. Doddala, pers. obs.). Fresh undamaged leaves of test plants were used as host plant stimulus, and clean air was used as control.

Intraspecific differences in olfaction were tested, using Havelock North apple and Waikanae blackberry populations that have different geographical and host plant association, by dual-choice bioassays (apple vs. blackberry) in the Y-tube olfactometer. In addition, two sets of feeding bioassays were conducted either as a dual-choice (apple and blackberry) or a tri-choice (apple, blackberry and bush lawyer) assay with the two test beetle populations. Bush lawyer (*Rubus australis*) is a New Zealand native Rosaceae that has never been reported as a host plant for the beetles; we included this plant to test how a novel host would influence meal composition. All the experiments were conducted during the active seasons (New Zealand spring – summer) 2009–2013.

### Olfactometry

A glass Y-tube (lateral arms 22.2 cm long and 4.5 cm diameter; central arm 24.2 cm long and 4.5 cm diameter) olfactometer was used for the host plant attraction bioassays. The two arms of the Y-tube were connected to an airflow meter, which in turn was connected to a compressed air source with an activated charcoal filter. Air was passed through the two arms of the Y-tube simultaneously at equal pressure and into the main central arm at a rate of 400 ml/s.

Individual beetles (one at a time) were released into the opening of the central arm of the Y-tube through a specimen chamber (4 cm diameter) and exposed to two different treatments placed in the treatment chambers of the two lateral arms. The two chambers (4 cm diameter) containing treatment choices were separated from the Y-tube by a sterile black fabric as a visual barrier and to prevent beetles from entering the choice chambers. Beetles were given a maximum of 20 min in the Y-tube to make a choice. A positive

Post-feeding, the leaf discs were photographed using an Olympus Camedia C-5050 fitted to an Olympus SZX-ILLD2-200 dissecting microscope. Photographs were analysed using the leaf area analysis software, Compu-eye (Bakr 2005) to calculate the area consumed. The leaf discs were weighed again after the bioassay and leaf mass consumed was calculated as the difference between the initial and final weight of the leaf disc.

### Data analysis

The total number of beetles that responded in the olfactometry was compared to the non-responsive ones by logistic regression and non-responsive beetles omitted from further statistical analysis. The number of beetles attracted to each of two options (test plant & air or test plant1 & test plant2) in the olfactometer bioassays was compared by binary logistic regression. Response index (RI) was calculated as follows.

$$RI_{\text{Option1}} = \frac{\text{Number of beetles attracted to Option 1}}{\text{Number of beetles attracted to Option 1} + \text{Number of beetles attracted to Option 2}}$$

response was scored when a beetle reached the end of an arm, at which point the run was deemed completed. Beetles that failed to choose any treatment in 20 min were recorded as non-responsive and eliminated from further analysis. Each beetle was used only once in the bioassays (replicated with 32–68 beetles per bioassay). After each run, the test beetle was removed, and the Y-tube was rinsed with n-hexane and air-dried before being used in the next bioassay and oven-dried at the end of each day. All the olfactometer experiments were conducted under artificial light at controlled conditions (20°C and 70% RH).

### Feeding bioassays

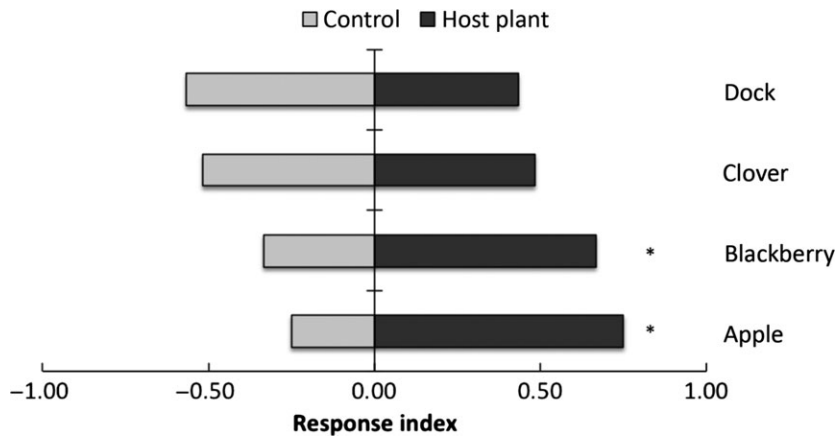
Leaf discs of 5 mm diameter were cut from fresh leaves of test plants; one leaf disc each of apple and blackberry or apple, blackberry and bush lawyer were presented to each test beetle in a Petri dish (5.5 cm diameter) lined with a moist filter paper (Whatman® Cat No 1001-055). Leaf discs were weighed prior to the bioassay. Each Petri dish was then sealed with cellulose tape (Sellotape®) to retain moisture. The beetles were allowed to feed on the leaf discs for 24 h, under controlled conditions (20°C, 70% RH) and 14-h:10-h light:dark regime, before being removed.

Response index for option 2 was also calculated, in the same way as shown above for option 1. Response indices for control in host plant vs. air assays are arbitrarily shown as negative values (fig. 1), whereas in apple vs. blackberry assays, response indices for blackberry are arbitrarily shown as negative values (fig. 2). The leaf area and leaf biomass consumed by individual beetles in the feeding bioassays were compared using ANOVA followed by Tukey's HSD multiple comparisons if the overall F-test was significant. Acceptance and rejection of a host plant in feeding assays were analysed by binomial exact test. Normality of leaf area and leaf mass were assessed with SAS Proc Univariate prior to analysis. SAS v.9.2 (SAS Institute Inc., Cary, NC) was used to perform all statistical analyses.

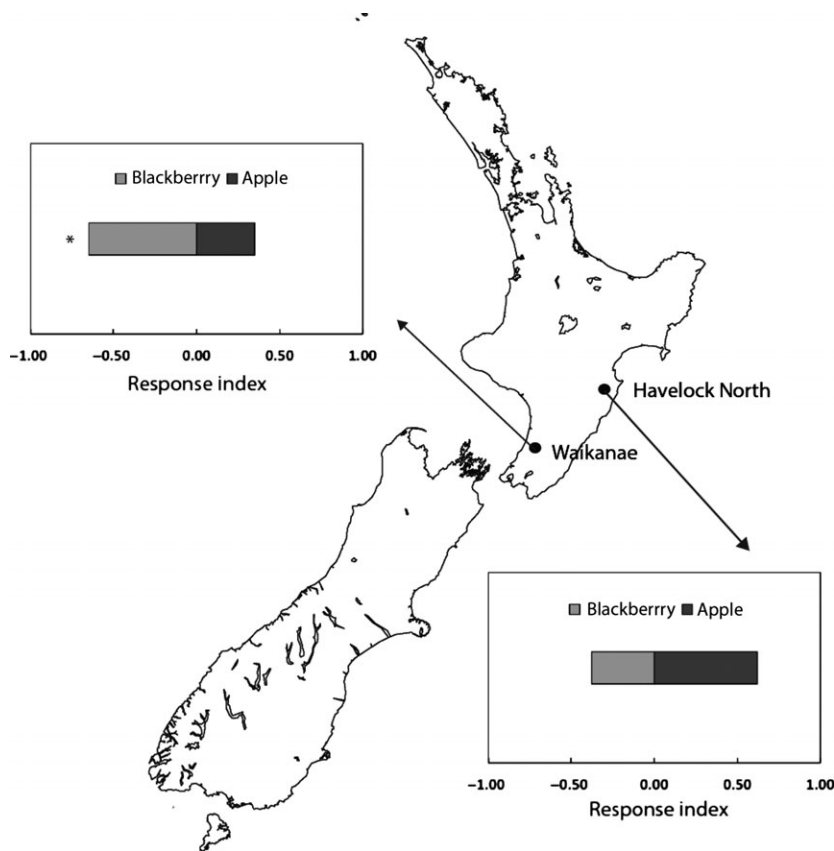
## Results

### Olfactory assays

Most of the test beetles moved (mostly walking with occasional short flights) away from the release point and towards odour/air source in all of the olfactometer bioassays (overall response in host plant vs. air



**Fig. 1** Attraction of adult *Eucolaspis* sp. beetles to host plant odour (apple/blackberry/white clover/broad-leaved dock) or clean air (control) in Y-tube olfactometer bioassays. \*P < 0.05. X-axis shows response index; positive response index indicates attraction.



**Fig. 2** Attraction of adult *Eucolaspis* sp. beetles from 'Havelock North apple' and 'Waikanae blackberry' populations to host plant odours (apple/blackberry) in Y-tube olfactometer bioassays. \*P < 0.05. X-axes denote response index; positive response index indicates attraction to apple; negative response index indicates attraction to blackberry. Map shows geographical locations of the two test populations (Havelock North and Waikanae) in the North Island of New Zealand.

bioassays was 87.3%; Wald  $\chi^2_{(1, N = 150)} = 61.86$ ,  $P < 0.001$ ). Males (88.3%) and females (86.4%) were equally responsive (Wald  $\chi^2_{(1, N = 150)} = 0.14$ ,  $P > 0.05$ ).

When a host plant odour was tested against control, both apple (Wald  $\chi^2_{(1, N = 36)} = 8.15$ ,  $P = 0.0043$ ) and blackberry (Wald  $\chi^2_{(1, N = 36)} = 3.84$ ,  $P = 0.049$ ) were attractive to the beetles, whereas white clover (Wald  $\chi^2_{(1, N = 29)} = 0.03$ ,  $P = 0.85$ ) and broad-leaved dock (Wald  $\chi^2_{(1, N = 30)} = 0.55$ ,  $P = 0.46$ ) were not (fig. 1).

There were no sex-specific differences in the degree of attraction to a treatment/control in all the four bioassays ( $P > 0.05$ ).

When a choice of two favoured host plant odours (apple and blackberry) was offered to beetles from two different host populations, a significant interaction was observed between population source and host plant (Wald  $\chi^2_{(2, N = 110)} = 15.94$ ,  $P < 0.001$ ). There was a preference towards parental host plant. A total of 62% and 65% of test insects from Havelock

North apple and Waikanae blackberry populations, respectively, recognized their parental host plant – but only Waikanae blackberry population showed statistically significant attraction (Wald  $\chi^2_{(1, N = 60)} = 5.2$ ,  $P = 0.022$ ) (fig. 2). There was no effect of gender in the degree of attraction (Wald  $\chi^2_{(2, N = 110)} = 0.87$ ,  $P = 0.65$ ).

### Feeding assays

Beetles of both Havelock North apple and Waikanae blackberry populations preferred to consume blackberry leaves rather than apple leaves (fig. 3a,b). The proportion of leaf area ( $F_{(1, N = 158)} = 13.04$ ,  $P < 0.001$ ) and amount of leaf mass ( $F_{(1, N = 158)} = 8.85$ ,  $P < 0.01$ ) consumed were significantly higher for blackberry in apple–blackberry choice. Waikanae blackberry beetles consumed less leaf irrespective of the host plant, compared with Havelock North apple beetles ( $F_{(1, N = 158)} = 68$ ,  $P < 0.001$  for leaf area), but this was driven by difference in quantity of consumption (both leaf area and leaf mass) and not by differences in preference between host plants (fig. 3b). Waikanae blackberry beetles displayed strong food preferences for blackberry – very few of the 31 test beetles fed on apple, preferring instead to feed exclusively on blackberry. Although many ‘Havelock North apple’ beetles fed on both apple and blackberry leaf discs, about 66% of the total leaf consumed was blackberry (fig. 3b).

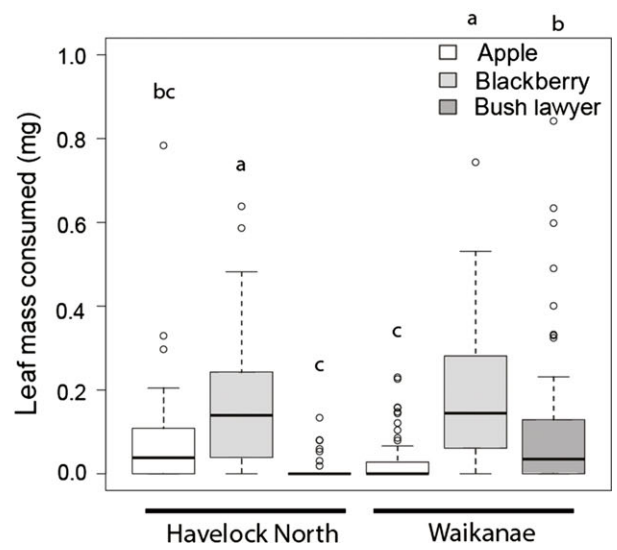
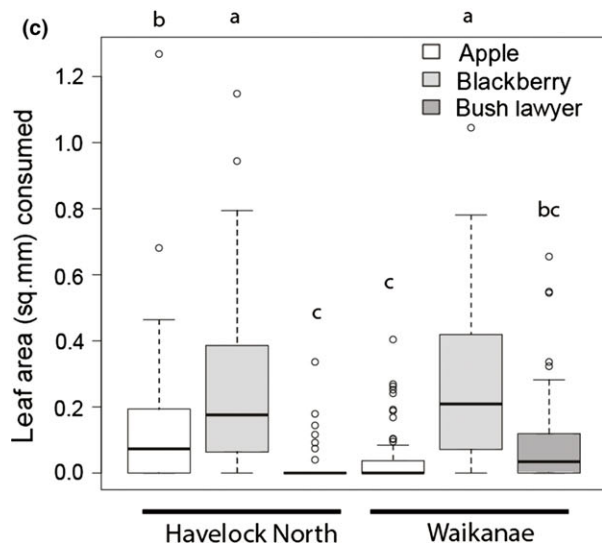
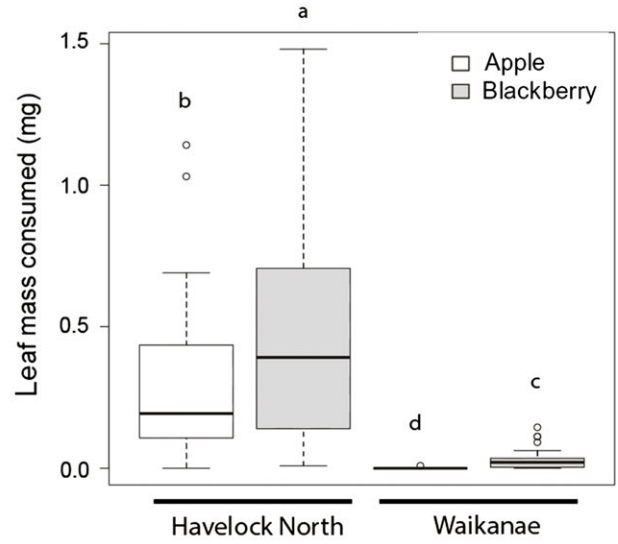
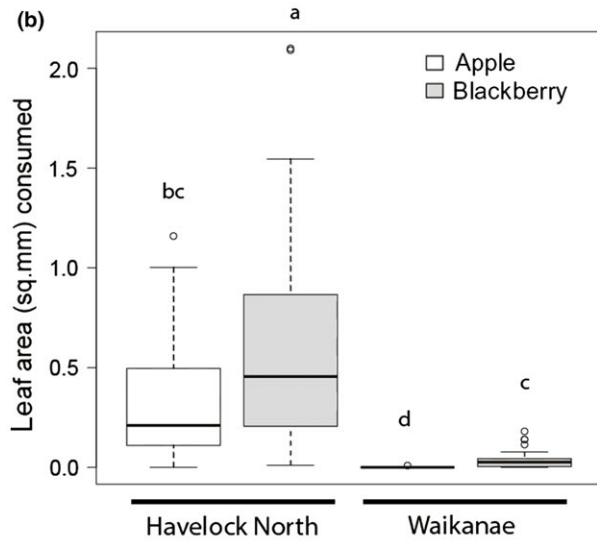
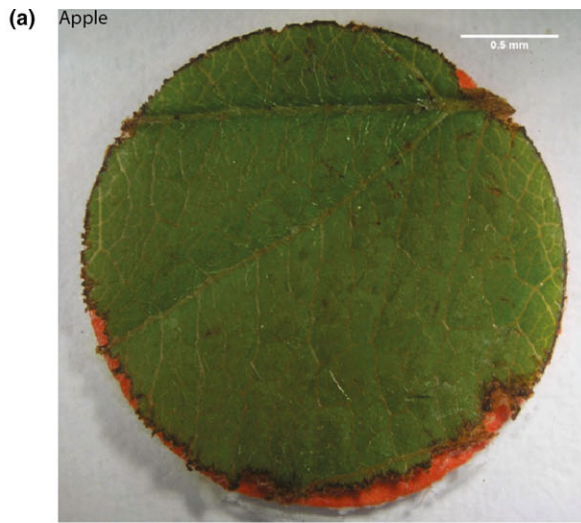
When a third novel native plant (bush lawyer) was introduced into diet choices, beetles fed on the bush lawyer to some extent. Beetles from Havelock North apple population did not show any preference between apple and bush lawyer, but preferred blackberry to both of these plants (fig. 3c). About half of the test beetles from Havelock North apple population rejected both apple and bush lawyer, whereas only few (12 out of 63 tested) rejected blackberry (binomial exact test,  $P < 0.01$ ). Beetles from Waikanae blackberry population showed different food choices compared with those of Havelock North apple population; their overall preference was blackberry > bush lawyer > apple (fig. 3c). The preferences were reflected in rates of acceptance or rejection of each plant (apple: 37% accepted,  $P = 0.51$ ; blackberry: 86% accepted,  $P < 0.01$ ; bush lawyer: 56% accepted,  $P = 0.29$ ). There was no effect of gender on feeding preferences.

### Discussion

Adult *Eucolaspis* sp. ‘Hawkes Bay’ beetles were attracted to odours from both apple and blackberry

when each was offered alone. Our field observations indicate that these plant species are the most intensely used hosts. Insects move towards odour sources in the field to find familiar host plants (Visser 1986), and strong upwind movement shown by adult bronze beetles in our study represents a likely odour-oriented host-finding behaviour in the field. Attraction of herbivorous insects to odours from apple plants is a widely documented phenomenon. Odours from apple leaves and fruitlets attract a range of orchard pest insects including apple maggot fly *Rhagoletis pomonella* (Fein et al. 1982), codling moth *Cydia pomonella* (Coracini et al. 2004), apple fruit moth *Argyresthia conjugella* (Bengtsson et al. 2006) and oriental fruit moth *Cydia (Grapholita) molesta* (Piñero and Dorn 2007). Likewise, blackberry plant volatiles attract insect herbivores such as aphids *Sitobion avenae* and *S. fragariae* (Lilley and Hardie 1996). Attraction to multiple host plants as evidenced in bronze beetles in present study has also been demonstrated in four species of rolled leaf beetles, *Cephaloleia dorsalis*, *C. erichsonii*, *C. fenestrata* and *C. placida* (García-Robledo and Horvitz 2009), which were attracted to plants belonging to families Marantaceae, Costaceae and Zingiberaceae. For some leaf beetles such as *Altica engstroemi* (Chrysomelidae: Alticinae), olfaction is less important than vision in locating their host plants (Stenberg and Ericson 2007). In our experimental setting, *Eucolaspis* sp. ‘Hawkes Bay’ beetles were not attracted to either white clover or dock by olfaction, although in the field the beetles were observed to feed on both these plants, at least in apple orchards. It is possible that the bronze beetles do not have olfactory abilities to recognize these two plants and use them as opportunistic hosts.

Our two test populations differed in their olfactory responses and there was a significant interaction between population source and host plant (fig. 2). Havelock North apple beetles did not show any preference between apple and blackberry when both plants were offered at the same time (fig. 2), but were attracted to each of these plants when offered alone (fig. 1), indicating that olfactory cues alone could be insufficient for host preference when otherwise attractive hosts are present together. On the contrary, the Waikanae blackberry beetles showed increased attraction to blackberry over apple (fig. 2), suggesting olfaction alone can lead to host choice of this beetle population. In some cases, olfactory cues alone may be insufficient for the insects to select a particular host plant, in which case other plant cues are used as well. When additional cues (tactile, gustatory and visual cues) were available in the feeding bioassays, the



**Fig. 3** (a) An example of leaf area of apple and blackberry consumed by *Eucolaspis* sp. beetles from the 'Havelock North apple' population in the feeding bioassay (relative feeding by a single beetle; both leaf discs were offered simultaneously). (b) Apple and blackberry leaf area (left) and leaf mass (right) consumed by adult *Eucolaspis* sp. from two different host plant populations ('Havelock North apple' population and 'Waikanae blackberry' population) in two-choice feeding bioassays. (c) Apple, blackberry and bush lawyer leaf area (left) and leaf mass (right) consumed by adult *Eucolaspis* sp. beetles of 'Havelock North apple' and 'Waikanae blackberry' populations, in three-choice feeding bioassays.

Havelock North apple beetles preferred blackberry over apple (fig. 3). It is possible, that the beetles, in addition to plant odours, use either visual cues, as in pine weevil *Hylobius abietis* (Björklund et al. 2005) or contact cues, as in tortoise beetle *Cassida canaliculata* (Heisswolf et al. 2007) to select host plants. The acceptance or rejection of a host plant upon contact could depend on differences in leaf morphology and chemistry, and the extent of feeding on a particular plant is largely governed by phagostimulants (carbohydrates, amino acids and other nutrients) and deterrents (plant secondary metabolites such as alkaloids) (Bernays and Chapman 1994). Even though we have not analysed nutritive composition of blackberry and apple leaves, blackberry leaves provided a more favourable diet regime than apple leaves for the bronze beetles as evident from this study (fig. 3). The intraspecific differences between populations, as observed in the present study, are in line with observed behaviour for several other chrysomelids. For example, the feeding preference of willow-feeding leaf beetles, *Phratora vitellinae*, *Plagioderma versicolora*, *Lochmaea capreae* and *Galerucella lineola* on different cultivated and wild willow (*Salix*) species was influenced by leaf trichome density (Soetens et al. 1991) and composition of phenolic glycosides in the leaves (Tahvanainen et al. 1985). Similarly, chrysomelids *Acalymma* sp. and *Diabrotica* sp. preferred the cucurbitacin-containing squash variety 'Ambassador' over a squash variety 'Early Summer crook-neck' that does not contain cucurbitacin, and over non-cucurbitaceous plants such as corn and soya bean (Eben et al. 1997).

Chemoreception (olfactory and gustatory) plays a vital role in local adaptation of insect herbivores and may, in turn, act as a key driver for ecological speciation (Smadja et al. 2012). Accordingly, individual and population differences in host plant use could lead to formation of host races as in apple maggot fly (Jiggins and Bridle 2004) or show differences in host preferences even without apparent genetic differences as in *Helicoverpa* (Jallow et al. 2004). Differences between populations in preference to parental host plants could be due to differences in evolutionary history (Zvereva et al. 2010); in the current study, the Waikanae blackberry beetles may have had much longer evolutionary association with their

parental host plant than the Havelock North apple beetles. Affiliation to specific host plants in geographically isolated conspecific populations is not uncommon in Chrysomelidae, but so far majority of the evidence is pertaining specialist leaf beetles. For example, host plant preferences varied within populations of *Chrysomela lapponica* (Chrysomelidae), with individual populations preferring different species of willow (*Salix*) (Zvereva et al. 2010). Similarly, different locality populations of *Agelasa nigriceps* (Chrysomelidae) show varying levels of acceptance towards a recently established host plant, *Pterostyrax hispidus* (Kohyama et al. 2012).

An interesting finding from our study is the readiness of beetles to accept bush lawyer as a host. Although there is no information on how bush lawyer affects individual and population fitness, the majority (>50%) of the test beetles fed on it in our laboratory assays (fig. 3c). In the wild, the suitability of a host plant would also depend on habitat suitability for the immature stages. While *Eucolaspis* adults feed on a variety of mainly broad-leaved host plants (Table S1), larvae feed on roots of grasses (Kay 1980). Bush lawyer was preferred over apple by beetles of the Waikanae blackberry population (fig. 3c), suggesting an innate adaptation for closely related plant species (bush lawyer and blackberry are different species of the same genus, *Rubus*).

Blackberry, a common weed with distribution throughout New Zealand (Popay et al. 2010), could act as a reservoir of bronze beetles that invade into economically important fruit crops such as organic apple orchards. However, it is not known how far bronze beetles can disperse, and it has been shown that the full reproductive cycle can be completed within orchards. Although adjacent cropping activity was found to have no influence on bronze beetle density within infested apple orchards (Rogers et al. 2006), fresh invasions may be common owing to attraction of beetles to apple volatiles. The relationship between native and exotic hosts of *Eucolaspis* beetles is still obscure. Many native hosts are known (Table S1), but as yet there are few data demonstrating the rate of host switching among these beetles. Our finding that the beetles readily accept a novel host (bush lawyer) offers future scope for exploring the dynamics of host-switch.

## Conclusions

We establish the first evidence of utilization of host plant volatiles in host location by an Eumolpinae taxon. In their natural environment, bronze beetles may use other cues in addition to plant volatiles; but this study demonstrates that the beetles locate hosts even when only olfactory cues are provided. We also show, from our olfactory and feeding bioassays (figs 2 and 3), that olfaction plays an important role in local adaptation. However, other cues may override the olfactory input (at least in the Havelock North beetle population) and the local adaptation is rather dynamic and may depend on evolutionary history. Olfaction may lead insects to potential host plants, but final acceptance or rejection of a particular host may also involve other sensory pathways (visual, tactile and gustatory).

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** A list of host plants of adult *Eucolaspis* in New Zealand.