Identifying plant DNA in the faeces of a generalist insect pest to inform trap cropping strategy

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Abstract

Monocropping elevates many insects to the status of economic pests. In these agroecosystems, non-crop habitats are sometimes deployed as trap crops to reduce pest damage. This environmentally friendly alternative to pesticides can be particularly fitting when dealing with native invaders that may be afforded legal protection or enjoy public sympathy as is the case for the ground wētā *Hemidrus* sp. ‘promontorius’ (Orthoptera) in New Zealand. However, this approach requires knowledge of the insects’ diet to select the most appropriate plant species for trap cropping. Here, ingested plant DNA in the faeces of wētā was analysed to help develop strategies for mitigating its damage in New Zealand vineyards. DNA was extracted from faeces of wētā collected from six different vineyards over four seasons. Using a DNA metabarcoding approach, we amplified the *rbcL* gene region and sequenced the amplicons on an Illumina MiSeq platform. The identity of plants in the diet of this insect was determined by comparing the sequences generated with those available in the GenBank database and cross-checking the results with a database of plants known to be present in New Zealand. A total of 47 plant families and 79 genera were detected. Of the genera identified, *Vitis*, *Poa*, *Festuca*, *Anthoxanthum*, *Anagallis*, *Camelina*, *Epilobium*, *Menyanthes*, *Pedicularis*, *Urtica*, *Garrya*, *Pinus* and *Tilia* were the major ones (i.e. they were present in more than 50% of the faecal samples). The composition of the above plant taxa in faecal materials was significantly different between collection sites or dates, except for *Menyanthes*. The occurrence of the latter was significantly different between collection sites. These results indicate that effectively mitigating wētā damage to vines requires the use of a diverse mix of plant species for trap cropping as wētā seem to be highly generalist in their feeding behaviour even when plant diversity is relatively low.

Keywords Wētā · Diet analysis · DNA metabarcoding · Faeces · Pest management · *rbcL* · Vineyards · New Zealand

1 Introduction

Agricultural intensification has led to monocultures of high yielding plant species/cultivars over vast areas of land. This provides abundant resources for insects feeding on those monocultural species, elevating them to the status of

economic pests (Rusch et al. 2016). To reduce pest damage while maintaining a monocultural state, high amounts of inputs are often applied, especially prophylactic use of insecticides and herbicides. These practices have led to major biodiversity losses and unwanted adverse effects on arable land and the surrounding environment (Rockström et al. 2009). Although the risks to human health and the environment from these chemicals have resulted in some cases in shifts to more sustainable non-pesticide pest management practices (Ekström and Ekblom 2011), most food production worldwide still relies heavily on high-input practices.

Alternative strategies, although still under-deployed, have as a key component the enhancement of functional farmland plant diversity (Rusch et al. 2016). This is because areas of non-crop habitats in farmland can influence pest populations by harbouring pests’ natural enemies (Gurr et al. 2016). Non-crop vegetation in or around farmland may also attract, divert or intercept the targeted insect pest(s) and reduce their damage

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to the main crop. These latter processes include trap cropping as well as supplemental management strategies such as trap vacuuming, trap harvesting, sticky traps and pesticide application to trap crops (Moreau and Isman 2012).

These pest management principles have been used worldwide in a variety of cropping systems including viticulture (e.g. Villanueva-Rey et al. 2014). For instance, although vineyards are almost monocultures, it is common for at least one grass species to cover the inter-row areas. In addition, strips of flowering plants (e.g. buckwheat, *Fagopyrum esculentum* Moench.) are sometimes sown under vines or in the inter-rows to enhance populations and fitness of natural enemies for managing important vine insect pests such as larvae of the leafroller complex (*Epiphyas postvittana*, *Ctenopseustis* spp., *Planotortrix* spp., etc.), leafhoppers (*Erythroneura* spp.) and other phytophagous insects (Shields et al. 2016). Inter-row vegetation and any surviving weeds could also act as alternative food sources for generalist insect pests, thereby potentially reducing economic damage.

However, successfully using this approach to manage pests hinges on identifying and deploying appropriate non-crop species (Gurr et al. 2016). Hence, deployment of a less suitable non-crop vegetation will not result in reduced pest damage to the main crop (Villa et al. 2016).

Generally, identification of candidate trap-crop species may involve the time-consuming observation of the insect's feeding behaviour, or alternatively, analysing its gut content or faeces for the most abundant plant species (Pompanon et al. 2012). Several classical methods of gut content or faecal analysis are available (e.g. microhistological analysis, near infrared reflectance spectroscopy, stable isotopes etc.), but they often lack taxonomic resolution (Soininen et al. 2009). More recently, advances in DNA barcoding, combined with high-throughput DNA sequencing, allow for the identification and characterisation of the composition of an animal's diet with much higher precision (Soininen et al. 2009; Alberdi et al. 2019; Pompanon et al. 2012; Boyer et al. 2013).

Insects emerge as pest when they are introduced to a new habitat, just as the introduction of new crop plants can also lead to novel associations where native species become pests (Lefort et al. 2015). This is the case for one species of wētā which is native to New Zealand but has become a pest in vineyards (Nboyine et al. 2016). Wētā *Hemiandrus* sp. 'promontorius' are present in New Zealand's vineyards throughout the year and cause significant damage to vines at the specific period of budburst when they feed on the very young leaves (Nboyine et al. 2017).

Wētā are a well-known and iconic group of New Zealand insects comprising about 70 species in the families Anostomatidae and Rhaphidophoridae. Their name is derived from that of Wētāpunga, the god of ugly things in the Māori mythology. As such, these insects are considered *Taonga* (i.e. treasure) and must be protected. All wētā are

endemic to New Zealand and many of them are at risk of extinction because of the degradation of their natural habitat and the introduction of mammalian predators (in particular rats, mice and stoats). Because many species are threatened, wētā have become useful indicators of environmental health and the focus of numerous conservation initiatives. They are also ideal candidates for citizen science and science outreach projects because they are easier to work with than most other insects, they can be relatively large and are very appealing to the public. As a consequence, wētā are one of the only insect groups that is well recognised and highly valued by the general public.

Due to their endemic status, their significance in the Maori culture and the sympathy they generate from the public, it is not conceivable to control wētā populations with insecticides and alternative methods.

The current work therefore aimed at analysing ingested plant DNA in the faeces of a generalist orthopteran pest, a ground wētā (*Hemiandrus* sp. 'promontorius': Orthoptera, Anostomatidae), in New Zealand vineyards to help identify candidate plant families/genera for inclusion in its management strategy, for example, as potential trap plants. Using generic PCR primers, we anticipate to detect a range of plant taxa eaten by wētā and to obtain a good coverage of the insect's plant-based diet. According to the existing literature (e.g. Johns et al. 2001), we hypothesise that wētā feed not only on grass and vines but also on a number of plant species that may be less common in New Zealand vineyards. Seasonal variation in diet is expected as some of the targeted plants may only be available at certain periods of the year.

2 Materials and methods

2.1 Wētā collection sites

Six vineyard blocks located in three different vineyard locations were sampled in the Awatere Valley, Marlborough, New Zealand, at elevations ranging from 8 to 46 m asl; O-Block (Castle Cliffs, −41.6103 °S, 174.1276 °E) was 4.61 ha; D-Block (Castle Cliffs, −41.6075 °S, 174.1328 °E) was 37.88 ha; H-Block (Castle Cliffs, −41.6131 °S, 174.1359 °E) was 2.98 ha; L-Block (The Favourite, −41.6198 °S, 174.1071 °E) was 16.88 ha; N-Block (The Favourite, −41.6260 °S, 174.1105 °E) was 44.41 ha and CR-Block (Caseys Road, −41.6880 °S, 174.120 °E) was 11.98 ha. These vineyards were subjected to conventional management practices, with weeds, insect pests and diseases being controlled with pesticides. The inter-rows were densely sown with grass mixtures dominated by *Lolium perenne* L., *Festuca arundinacea* Schreb. and *Poa pratensis* L., while under-vine areas sometimes harboured a few sparsely growing dicotyledonous weeds and grasses. In spring, under-vine areas were

sprayed with conventional herbicides to remove weeds and maintain the soil bare. Pine tree (*Pinus* L. spp.) hedges bounded at least one side of each sampled block.

2.2 Sampling wētā from vineyards for faecal analysis

Sampling was performed during the day while wētā are generally buried in individual galleries (Fig. 1). On random locations in the vineyard, the upper layer of soil was swiftly removed with a movement of the shovel to expose galleries inhabited by wētā and draw the insects out. Wētā mid-instar larvae were hand-collected as they came out of their galleries. Each of the six vineyard blocks were sampled over four seasons, namely in July 2014, October 2014, January 2015 and April 2015. Sampling on one vineyard block took about 2 h and all blocks were sampled within 4 days at each season to avoid any difference in food availability due to plant phenology. In each season, 60 individual insects (i.e. 10 from each of the six vineyard blocks) were collected and placed singly in a labelled plastic arena (9 cm height × 15 cm width × 15 cm length) lined with a double layer of tissue paper. The arenas were stored at room temperature (20 °C) for 24 h, after which the insects were released. Individual wētā mostly produced one faecal pellet which was stuck to the tissue paper. Each pellet was carefully transferred into a labelled 60 mm diameter Petri dish (excluding the tissue) and stored at − 80 °C pending DNA extraction.

2.3 DNA extraction

DNA was extracted from 72 faecal samples (i.e. three randomly selected pellets per site per season) using a Zymo Research Fecal DNA MicroPrep™ kit. The manufacturer's protocol was followed with slight modifications. To extract DNA from wētā faeces, 500-μl lysis solutions were pipetted into 72

Fig. 1 Photograph of a female ground wētā *Hemiandrus* sp. 'promontorius' with egg clutch after excavation of her burrow

individual BashingBead™ lysis tubes each containing faecal material. The DNA from the faecal material produced by an individual wētā was extracted individually as its weight was less than the 150 mg recommended by the manufacturer. The tubes were secured in a bead beater and processed at 50 oscillations per second for 5 min, followed by centrifuging at 10,000 g for 1 min. The supernatants (400 μl) were transferred to Zymo-Spin™ IV spin filters in collection tubes and centrifuged at 7,000 g for 1 min. Faecal DNA binding buffer (1200 μl) was then added to the filtrates after which the resulting mixtures were transferred to Zymo-Spin™ IC columns in collection tubes and centrifuged at 10,000 g for 1 min. This was followed by the addition of 200-μl DNA pre-wash buffer and 500-μl faecal DNA wash buffer to the columns and centrifuging for 1 min at 10,000 g after adding each reagent. The columns were transferred into clean 1.5-ml microcentrifuge tubes and 30 μl of DNA elution buffer were added directly to each column matrix. The tubes were centrifuged for 30 s at 10,000 g to elute the DNA. The latter was transferred into Zymo-Spin™ IV-μHRC spin filters in clean 1.5-ml microcentrifuge tubes and left for 30 min before centrifuging at 8,000 g for 1 min for purification. The purified DNA was then amplified through polymerase chain reaction (PCR).

2.4 PCR and electrophoresis

The universal primer pair (rbcL19 and rbcLZ1 (Poinar et al. 1998)), which amplifies a ≥ 150 base pairs (bp) fragment of the ribulose biphosphate carboxylase large subunit (rbcL) chloroplast DNA gene region, was used to detect ingested plant DNA in wētā faeces. Primers were designed to include the recommended overhang adapters for Illumina sequencing. The PCR amplification was performed in 40-μl reaction mixtures containing 6-μl DNA extract, 6.8-μl water, 20-μl GoTaq® Green 2 × 2-μl bovine serum albumin (BSA, 10 mg/ml), 2-μl MgCl₂ (25mM) and 1.6 μl each of the forward and reverse primers (10 μM). The protocol for the thermocycling was 94 °C for 5 min, 45 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s, and a final elongation at 72 °C for 10 min. Positive (*Lolium perenne* DNA) and negative (PCR grade water and wētā DNA extract) controls were included in each PCR to check for the success of amplification and DNA contamination, respectively. All PCR products underwent gel electrophoresis to check for successful amplification. Products of expected fragment size were cleaned with an Agencourt® AMPure® XP PCR purification kit following the manufacturer's instructions and standardized at 2 ng/μL. This procedure was also applied to the wētā DNA negative control. Unique molecular identifiers (MID) were added to each of the 72 samples as well as the negative control before high-throughput DNA sequencing on one run of Illumina MiSeq using the 300 × 300 paired-end protocol as

recommended by the manufacturer (https://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html). A 600-cycle kit was used to sequence the amplicons on the MiSeq instrument. Read 1 was sequenced to 320 base pairs, and Read 2 sequenced to 280 base pairs. Identifier ligation and Illumina sequencing were performed by New Zealand Genomics Ltd, Auckland, New Zealand.

2.5 Data analysis

Paired-end reads were merged using the software VSEARCH version 1.9.5. For quality control reads were truncated at the first low-quality base (i.e. quality score < 3) if present to ensure high-quality tails and accurate merging of the paired-end reads. Merged sequences from the Miseq run that were shorter than 150 bp were discarded because the expected length was 210 bp (~ 150 bp for the internal amplicon plus 30 bp for each primer). At this stage, we discarded any sequence with more than one expected error in the sequence as well as singletons (i.e. operational taxonomic units (OTUs) represented by a single read). To make the downstream analysis faster, non-unique sequences were then collapsed with a one base mismatch allowance. These unique sequences were clustered into molecular operational taxonomic units (MOTUs) using a conservative 97% identity threshold. Chimeric sequences were then removed using the UCHIME de novo method. To determine the identity of plant taxa in the diet of wētā, each MOTU had its representative sequence searched against the GenBank nucleotide database using BLASTN version 3.2.31. Identifications accepted as correct matches and used for subsequent analyses in this study were those where BLAST search returned values of query coverage > 80% (i.e. identification based on at least 120 base pairs out of 150), and identity > 90% (i.e. identification at genus level). Because rbcL is not perfectly resolutive at the species level in plants and the DNA fragment used was very short, the risk of obtaining assignments that matched several different taxa with the same score was higher than that commonly encountered in DNA barcoding studies. To minimise the risk of multiple assignments, we conducted a barcoding gap analysis using the local minima function in the R package SPIDER (Brown et al. 2012) to determine the appropriate species identity threshold based on our own data. This analysis found the species identification threshold for our dataset to be 1.8% (Fig. 2). The accepted identifications were further cross-checked with a database of plants present in New Zealand (Allan Herbarium 2000). Sequences with no match in BLAST or with a match not recorded in the database of plants present in New Zealand were removed from the dataset and not used in subsequent analyses. See decision map in Fig. 2 for details.

Data were analysed as frequency of occurrence (FOO) and relative read abundance (RRA) (Deagle et al. 2019). To calculate FOO, the data were converted into presence (1)/absence

(0) before performing statistical analyses. To limit the potential inclusion of contaminants, a filtering step was also performed, in which 'presence' was assigned to MOTUs that occurred at least four times (i.e. 4 reads) in one faecal sample, while 'absence' was assigned to those that were detected less than four times and only present in one faecal sample.

Because our main interest was in the detection of food items that could potentially be used in a trap cropping strategy. Statistical analyses of FOO focused on major food items, which were defined as those genera which were detected in more than 50% of the faecal samples. These major taxa were *Vitis* sp. (vines), *Poa* spp. (grass), *Festuca* spp. (grass), *Anthoxanthum* spp. (grass), *Anagallis* spp. (weed), *Camelina* spp. (weed), *Epilobium* spp. (weed), *Menyanthes* spp. (weed), *Pedicularis* spp. (weed), *Urtica* spp. (weed), *Garrya* spp. (tree), *Pinus* spp. (tree) and *Tilia* spp. (tree).

Food items were categorised in two groups: 'cultivated' plants, when grown for economic reasons (vines) or to provide other beneficial services such as erosion control (grasses), and 'uncultivated' plants, which were weeds and trees growing inside or outside the vineyards, respectively.

Accumulation curves were built based on the cumulative number of plant families and genera detected in relation to the number of samples analysed using a bootstrap method to estimate diet coverage. Generalised linear models were used to determine the effect of sites and dates of sampling on the detection of each of the eight major taxa. The binomial distribution (with a binomial total of three faecal samples for each sampling unit) and logit link function were chosen for these analyses. The response variables were the diet (i.e. the eight major plant items), while the fitted model comprised date and site. Main effect means for either date or site that were significantly different were separated using least significant differences (LSD). Significant differences between the proportions of groups, subgroups and genera of plants were determined by computing the 95% confidence intervals (C. I.) of their mean. Relative read abundance was compared between sites and season using an analysis of deviance on a multivariate generalised linear model. A negative binomial distribution was chosen for this analysis based on the dispersion of the residuals. A 5% probability level was used for all tests.

3 Results and discussion

A total of 7,413,745 quality reads were obtained, of which 7,026,022 reads provided quality matches with 540 MOTUs from GenBank. Applying a species identification threshold of 1.8% resulted in 40 MOTUs identified at species level and 500 that could only be identified at genus level. Of the latter, 366 MOTUs (i.e. 5,024,502 reads) corresponded to genera known to be present in New Zealand (Fig. 2). Overall, 80% of plant taxa present in the faecal materials could be

Fig. 2 Decision tree and number of reads and MOTUs retained or discarded at each step of the bioinformatics analysis

confidently identified at the genus level, while only 3% could be identified at species level. The *rbcL* gene was therefore resolutive enough for genus level identification of plants, making it particularly suitable for studies focusing on ecosystems with moderate levels of plant biodiversity such as intensive agricultural landscapes. Based on these results, data was analysed only at the genus and family level and the following analyses were based on a total of 401 MOTUs represented by 6,129,201 reads (green boxes on Fig. 2).

In the current study, the *rbcL* gene region was targeted using general primers. A major advantage of using a general primer set is that a priori knowledge of the range of potentially consumed species (i.e. taxonomic coverage) by herbivores is not required (Pompanon et al. 2012). In such single-locus studies, the P6 loop of the *trnL* intron or the *rbcL* region are usually recommended because these regions are easily amplified and are well conserved in land plants, thus allowing to achieve a high taxonomic resolution when using a metabarcoding approach (Alberdi et al. 2019; Pompanon et al. 2012). In addition, these regions are relatively short (ranging between 12–134 bp and 150 bp respectively), which makes them more likely to be amplified from degraded DNA samples such as faeces and gut contents (Pompanon et al. 2012). In the New Zealand context, *rbcL* sequences are available for the great majority of native and naturalised plant genera (Lear et al. 2018), which makes it the better candidate. A recent study by McClenaghan et al. (2015) successfully described the diet of different species of grasshoppers (Orthoptera: Acrididae) in Ontario by using the same primers

and identifying plants at the family level and to a lesser extent, at genus and species levels.

The identified taxa belonged to 47 plant families and 79 genera. According to a bootstrapped estimate using good quality sequences from 72 faecal samples, an estimated 93.7% of all plant genera and 96.4% of all plant families likely present in the diet of the wētā were successfully detected. Our analysis was therefore sufficient to determine the overall diet of that species at these taxonomic levels. Of the families detected, Poaceae comprised 12 genera, while the families Amaranthaceae, Asteraceae, Podocarpaceae and Rosaceae recorded four genera each. Except Lamiaceae, the remaining 41 families displayed at most two genera (Table 1). Overall (i.e. irrespective of sampling site or season) the genera *Vitis*, *Poa*, *Festuca*, *Anthoxanthum* and *Tilia* were more frequently detected than any other taxon (Fig. 3a).

The mean detection rate of cultivated plants (grasses and vines) ($P < 0.05$; C. I. = 0.98–1) were significantly higher than that of uncultivated plants (weeds and trees) ($P < 0.05$; C. I. = 0.67–0.75). Pairwise comparisons of the mean proportional detections of the different categories of plants showed that vines and grass (*Poa* sp., *Festuca* spp., *Anthoxanthum* spp.) occurred more often than trees (*Pinus* spp., *Tilia* spp., *Garrya* spp., etc.) which were detected more often than dicotyledonous weeds (*Anagallis* spp., *Camelina* spp., *Epilobium* spp., *Menyanthes* spp., *Pedicularis* spp., *Urtica* spp., etc.) (Fig. 3c). It is important to note that weeds were rare in the vineyards studied (J. Nboyine, pers. Observ.). However, every wētā collected had eaten at least one of these weed species in

Table 1 Plant taxa identified from wētā faeces and their detection rate (i.e. proportion of wētā faecal material tested positive for each taxon)

Family	Genera detected	Common name	Detection rate (%)
Alstroemeriaceae	<i>Luzuriaga</i> Ruiz & Pav. 1802	-	27.42
Amaranthaceae	<i>Amaranthus</i> L.	Amaranth	24.19
	<i>Atriplex</i> L.	Saltbush	9.68
	<i>Chenopodium</i> (L.) S. Fuentes, Uotila & Borsch	Nettle-leaved goosefoot	3.23
	<i>Suaeda</i> Forssk. ex J. F. Gmel.	-	35.48
Amaryllidaceae	<i>Allium</i> L.	Onion	4.84
Apocynaceae	<i>Catharanthus</i> G. Don	Periwinkles	8.06
Asparagaceae	<i>Chlorophytum</i> Ker Gawl.	Spider/airplane plant	8.06
	<i>Cordyline</i> Comm. ex R.Br.	Cabbage-palm	1.61
Asteraceae	<i>Crepis</i> L.	Narrowleaf hawksbeard	3.23
	<i>Guizotia</i> Cass. 1829	Annual herb	8.06
	<i>Senecio</i> L.	Ragworts	17.74
	<i>Tragopogon</i> L.	-	1.61
Betulaceae	<i>Ostryopsis</i> Decne.	-	4.84
Boraginaceae	<i>Cordia</i> L.	Shrubby tree	41.94
Brassicaceae	<i>Camelina</i> Crantz	False flax	74.19
	<i>Raphanus</i> L.	Radish	3.23
Calyceraceae	<i>Acicarpa</i>	-	4.84
Cannabaceae	<i>Celtis</i> L.	Nettle trees/hackberries	17.74
Caryophyllaceae	<i>Silene</i> L.	Catchfly	3.23
Ceratophyllaceae	<i>Ceratophyllum</i> L.	Hornwort	6.45
Convolvulaceae	<i>Ipomoea</i> L.	-	1.61
Cucurbitaceae	<i>Cucumis</i> L.	Muskmelon	11.29
Cupressaceae	<i>Cunninghamia</i> R.Br.	-	8.06
Cyperaceae	<i>Carex</i> L.	Sedges	1.61
Dennstaedtiaceae	<i>Hypolepis</i> Bernh. 1806	-	14.52
Fabaceae	<i>Alysicarpus</i> Desv.	Moneyworts	1.61
	<i>Lespedeza</i> Michx.	Bush clovers	1.61
Fagaceae	<i>Quercus</i> L.	Oak tree	30.65
Garryaceae	<i>Garrya</i> Douglas ex Lindl.	Ashy silktassel	87.10
Geraniaceae	<i>Erodium</i> Aiton	Redstem filaree	12.90
Goodeniaceae	<i>Scaevola</i> L.	Fairy fun-flower	1.61
Grossulariaceae	<i>Ribes</i> L.	Currants	1.61
Lamiaceae	<i>Nepeta</i> L.	-	22.58
	<i>Prunella</i> L.	Self-heal	30.65
	<i>Stachys</i> L.	Woolly hedgenettle	1.61
Lauraceae	<i>Machilus</i> Nees	-	14.52
Malvaceae	<i>Tilia</i> L.	Lime tree	93.55
Menyanthaceae	<i>Menyanthes</i> L.	-	70.97
Moraceae	<i>Morus</i> L.	Mulberry	12.90
Myricaceae	<i>Myrica</i> L.	-	12.90
Nothofagaceae	<i>Nothofagus</i> Blume		1.61
Onagraceae	<i>Epilobium</i> L.	Hoary willowherb	53.23
	<i>Oenothera</i> L.	Tall evening primrose	3.23
Orobanchaceae	<i>Pedicularis</i> L.	Warrior's plume	72.58
Pinaceae	<i>Cedrus</i> Trew	-	1.61
	<i>Pinus</i> L.	Pine	67.74
Poaceae	<i>Agrostis</i> L.	Bentgrass	43.55
	<i>Anthoxanthum</i> L.	Grass	95.16

Table 1 (continued)

Family	Genera detected	Common name	Detection rate (%)
	<i>Dactylis</i> L.	Grass	4.84
	<i>Eleusine</i> (L.) Gaertn.	Goosegrass	3.23
	<i>Elymus</i> L.	Grass	27.42
	<i>Festuca</i> L.	Fescue	100
	<i>Hordeum</i> L.	Grass	1.61
	<i>Oryza</i> L.	Rice	3.23
	<i>Paspalum</i> L.	Crowngrasses	48.39
	<i>Poa</i> L.	Grass	98.39
	<i>Sacciolepis</i> Nash	Cupscale grass	6.45
	<i>Sporobolus</i> R.Br.	Grass	1.61
Podocarpaceae	<i>Dacrydium</i> Lamb.	-	30.65
	<i>Halocarpus</i> C. J. Quinn	Tree	1.61
	<i>Nageia</i> Gaertn, 1788	Shrub	4.84
	<i>Podocarpus</i> L'Hér ex Pers. 1807	-	4.84
Polygonaceae	<i>Fagopyrum</i> Mill.	Buckwheat	14.52
	<i>Polygonum</i> L.	Japanese knotweed	24.19
Primulaceae	<i>Anagallis</i> L.	Pimpernel	59.68
Prumnopityaceae	<i>Prumnopitys</i> Phil.	-	1.61
Ranunculaceae	<i>Ranunculus</i> L.	Buttercup/crowfoot	3.23
Rhamnaceae	<i>Ceanothus</i> L.	-	9.68
Rosaceae	<i>Fragaria</i> L.	Strawberries	30.65
	<i>Potentilla</i> L.	Cinquefoils	1.61
	<i>Prunus</i> L.	Bitter berry	22.58
	<i>Rubus</i> L.	Raspberries, blackberries	3.23
Salicaceae	<i>Populus</i> L.	-	24.19
Solanaceae	<i>Ichroma</i> Benth.	Shrub	20.97
	<i>Solanum</i> L.	Tomato	19.35
Urticaceae	<i>Urtica</i> L.	Annual nettle	69.35
Violaceae	<i>Viola</i> L.	Crow-foot violet	19.35
Vitaceae	<i>Vitis</i> L.	Grape vine	100

spite of the unlimited availability of grasses and vines. A similar pattern was observed for trees (*Pinus* spp., *Tilia* spp, *Garrya* spp., etc.), which were also represented in every faecal sample. The high diversity of plant families and genera identified from the faecal samples confirmed the status of this wētā as a generalist feeder. Species in the genus *Hemiandrus* are usually omnivores, feeding not only on a diverse range of plants including green leaves of trees and shrubs but also on other invertebrates (Van Wyngaarden 1995; Johns 2001). Diets comprising a mixture of plant and/or animal species are a common feeding behaviour among generalist orthopterans and other omnivore arthropods (Coll and Guershon 2002). This gives such insects a better nutrient balance than is possible by feeding on a single plant taxon, resulting in increased growth and survival (Coll and Guershon 2002; Berner et al. 2005). In addition, toxic secondary metabolites produced as defence mechanisms against herbivory by some

plant species are diluted in mixed diets, reducing their effect on the insect (Ali and Agrawal 2012).

With regard to the relative abundance of reads (RRA), 45% of all reads belonged to Poaceae and 41% to vines leaving only 14% of reads to the 31 remaining families (Fig. 4). These percentages reflect the composition of plants communities generally observed in the studied vineyards. The inter-rows of the vineyards studied were dominated by grasses, which are low in protein content (below 50% of DM) and high in carbohydrates. As the grasses mature, protein content declines to less than 10% while carbohydrate increases (Lledó et al. 2015). Proteins are a major requirement of the diet of *Hemiandrus* spp. (Johns 2001). Being an omnivore, this insect could balance its protein intake by preying on other insects. The latter were however killed by the regular applications of insecticides in the vineyard. Therefore, sustainable intake of protein for this wētā appeared to rely on balanced feeding on

Fig. 3 Detection of plant DNA through molecular analysis of frass from wētā (*Hemiandrus* sp. 'promontorius'). **a** Proportion of the major plant genera detected. Data are means $\pm$ 95% confidence intervals. Bars with no letters in common are significantly different at the 5% probability threshold. **b** Proportion of wētā frass tested positive for *Menyanthes* spp. at different sites sampled. Data are means $\pm$ standard error of means (S. E.). Bars with no letters in common are significantly different

at the 5% level of significance. **b** Proportion of the different plant categories detected in wētā frass. Data are means $\pm$ 95% confidence intervals. Bars with no letters in common are significantly different at the 5% probability threshold. Trees: *Pinus* spp., *Tilia* spp. and *Garrya* spp.; weeds: *Anagallis* spp., *Camelina* spp., *Epilobium* spp., *Menyanthes* spp., *Pedicularis* spp. and *Urtica* spp.; grasses: *Poa* spp., *Festuca* sp. and *Anthoxanthum* sp.; vines: *Vitis* sp.

weeds and tree species that have been detected, but because these were mostly rare in vineyards, it alternatively fed on vine buds. Hence, management practices that encourage patches of weed growth in vineyards could probably minimise wētā feeding on vines.

No dietary variation was detected in relation to date of sampling based on FOO and RRA (LRT = 126.8, $P = 0.262$). This was true when analysing the full dataset and when focusing on the major food items (plant genera detected in more than 50% of the samples analysed). Indeed, the proportions of faecal samples that tested positive for DNA of *Vitis*, *Poa*, *Festuca*, *Anthoxanthum*, *Anagallis*, *Camelina*, *Epilobium*, *Pedicularis*, *Urtica*, *Garrya*, *Pinus* and *Tilia* did not change significantly with date. This result is possibly due to a limited number of samples analysed for each season as cumulative curves show that the 18 samples analysed per season allowed detection of an estimated 60.6% of all plant genera and 80.5% of all plant families in the diet of wētā.

A significant difference was observed in relation to sampling location for RRA (LRT = 111.9, $P = 0.026$). In terms of detection, only *Menyanthes*, displayed a significant change in occurrence in relation to sampling location ($P = 0.028$).

Detection of this flowering annual weed in wētā faeces was highest in the O-Block and lowest at the N-Block. The detection rate of this genus in the D-, H- and L- Blocks was significantly lower than that recorded in the O-Block but higher than in the N-Block (Fig. 3b). These small geographical variations reflect slight differences in the blocks' plant communities and confirm that wētā are highly generalists and capable of feeding widely on the plants present in their environment.

Our study allowed for the identification of the main food items in the diet of wētā. This approach could be followed by food choice experiments to ascertain wētā food preferences between the small number of trap crop candidates identified here, and to select one or two optimal trap crops. Alternatively, and because wētā appear highly generalists, seeds of cultivars from various taxa could be sown in mixtures to provide divers alternative food for this insect. In this case, the main selection criteria may be the plant individual needs (climatic, soil, etc.) to ensure they provide enough resources at the critical time of vine budburst, thereby reducing damage to vines.

The deliberate use of weed species to attract natural enemies for insect pests' population regulation has been studied extensively (Sarkar et al. 2018). The findings here suggest that

Fig. 4 Wētā individual diet analysis. Relative read abundance (a) and occurrence (b) of plant genera as measured from each individual faecal sample. Frequency of occurrence (c) of the different plant genera, with major food items represented in colour

this approach to pest management (when adopted by vine growers) would have the added advantage of reducing crop damage by generalist insect herbivores and omnivores such as wētā, which may use weeds as alternative foods. However, to limit the multiplication of wētā, trap cropping should be

restricted to the vine sensitive period (around budburst) and the trap crop removed as soon as the vine leaves are tough enough that they are no longer targeted by wētā. Some unexpected genera were detected in the faeces analysed. For example, *Tilia* spp. (an ornamental mostly found in urban parks and

gardens), *Populus* spp., *Solanum* spp., *Ipomoea* spp., *Cucumis* spp., *Quercus* spp., etc. are not usually common around vineyards in New Zealand. Considering that the identified taxa were assigned up to the genus level, it is quite hard to anticipate the origin of those taxa in the samples analysed. Perhaps, they came from other sources. For example, because wētā are omnivorous and known to also feed on other insects, the presence of tree DNA may be explained by secondary predation. Further studies would be needed to clarify this observation. This notwithstanding, the molecular diet analysis used here highlighted the high level of diet mixing in this species and hinted of potential plant families or genera that can be used for trap cropping. Based on these results, effective management of wētā will likely require sowing more than one plant species as trap crops to adequately satisfy the nutritional needs of this insect. The proposed method also presents some limitations. Firstly, the use of a short single-locus molecular marker only allowed identification of most plant MOTUs at the genus level and gives no information about predation on other invertebrates. This last point is important because prey nutrient content can modify omnivorous insect's propensity to engage in herbivory (Ugine et al. 2019). Secondly, the RRA may not be an accurate quantitative measure of actual amount of each species consumed (Deagle et al. 2019). Thirdly, there may be poor representations of particular species for which primer affinity was low (Alberdi et al. 2019). Nonetheless, this type of analysis provides extremely valuable information, and as NGS technology improves, some of the above limitations are becoming less problematic (see Alberdi et al. 2019; Pompanon et al. 2012).

4 Conclusion

In summary, the current work examined how the results of faecal DNA analyses could potentially contribute to developing trap cropping strategy for managing a generalist insect pest, thus reducing the high pesticide input in most modern agriculture. Primers targeting a short fragment of the *rbcL* gene region were used to successfully identify the range of plants eaten by wētā, at least to the genus level. A wide variety of plant families were found in the diet of the target insect, in spite of grasses being abundant in vineyards. Such feeding behaviour is common among generalist insects, both herbivores and omnivores, and it is thought to ensure a balanced intake of major nutrients (proteins and carbohydrates). Hence, non-pesticidal management strategies for generalist insect pests could rely on trap crops that offset existing nutrient imbalances. For wētā, non-crop species with high protein content are recommended in agricultural systems dominated by plants with high carbohydrate content, and they should be planted to coincide with periods of damage to the economic crop. If these plants are potential weeds, they can be removed, for example

with herbicides, once the main period of pest-induced damage has passed. The method used here could be applied to other agricultural pests, in particular, those feeding on seasonal resources. By collecting insects and analysing their diet outside their damaging period, it is possible to acquire the necessary knowledge to apply an efficient trap cropping strategy. Many non-crop plants in vines or other crops deliver a wide range of ecosystem services throughout the growing season (Shields et al. 2016), some of which are not well understood. Managing non-crop plants in agriculture is key to achieve 'sustainable intensification' and could be better informed through molecular diet analyses of pest species.

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Statement of data availability Data and analyses (R codes) are available on a Figshare repository (doi: 10.6084/m9.figshare.5777853).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any study with animals performed by any of the authors.

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