Integrated management of ground Wētā (Orthoptera: Anostostomatidae) in Marlborough vineyards

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Entomology.

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The intensification of agriculture has led to monocultures of high-yielding plant species/cultivars over large areas of land. This provides abundant resources for insects which feed on those monocultural species, elevating them to the status of econmic pests. In the Marlborough region, New Zealand, the conversion of native vegetation in the Awatere Valley to pastures, and in the last 30 years to vineyards, has elevated an endemic orthopteran insect, referred to as wētā (Anostostomatidae) in Maori language, to occasional pest status. This wētā damages vine buds at budburst, consequently reducing yields. Damage is currently managed by tying plastic sleeves around the trunks of vines (*Vitis vinifera* L.); the sleeves are slippery and deny wētā access to buds. This management approach was adopted, instead of using pesticides, because of the significance of wētā in Maori culture and threats to populations of some wētā species. However, this management technique is labour intensive and costly, and sleeves often need to be repaired/replaced, leading to further costs. They also litter the environment when they become detached from the vines. Hence, this PhD work aimed at developing an ecologically-based integrated management strategy for wētā based on an understanding of the biology and ecology of the species associated with vine damage. A range of laboratory and field experiments were conducted to 1) confirm the identities and number of wētā species damaging vines, 2) wētā biology, densities and distribution in vine and non-vine habitats, 3) the range of plant species in wētā diet, 4) habitat manipulation strategies to mitigate wētā damage and 5) strategies to deter this insect from vineyards. A phylogenetic analysis of sequences obtained from wētā collected from vineyards confirmed that a single species was associated with bud damage. It was identified as *Hemiandrus* sp. 'promontorius' (Johns 2001) using morphological keys. This species is not threatened but has a restricted habitat range. It laid a mean of 55 eggs between March and May, and these hatched after five months. The sex ratio of this wētā was unity. Of three habitats searched, higher numbers of this insect per square meter were found in vines than in either pastures

or shrublands. Within vineyards, they were mostly found inhabiting burrows in the bare, moist and less compact soil under vines, with few wētā occupying burrows in the inter-row.

A high throughput analysis of DNA sequences from faecal pellets of wētā collected from vineyards showed that this insect feeds on plants from 30 families and 44 genera. Although vines and grasses were the dominant plants in the viticultural landscape studied, dicotyledonous weeds were found to be important components of wētā diet. In terms of management, three under-vine treatments [pea straw mulch (*Pisum sativum* L.), mussel shells (*Perna canaliculus* Gmelin, 1791), tick beans (*Vicia faba* Linn. var. *minor* (Fab.))] and two inter-row treatments [exisitng ryegrass-dominant vegetation, tick beans] were tested for their efficacy to mitigate wētā damage. Controls comprised vines with plastic sleeves (treated) or no sleeves (untreated), with the existing ryegrass-dominant inter-row vegetation. In this experiment, damage reduction resulted in a 28 and 39% significant yield increase in the under-vine bean and shell treatments respectively, compared to the untreated control. These yield increments were not significantly different from a 30% increment recorded in the sleeve treatment over the untreated control. Apart from mitigating wētā damage, some advantages of the under-vine bean and shell treatments over sleeve treatments include the ability of the beans to habour natural enemies for the control of other vine insect pests; shells conserve moisture and suppresses weed growth under the vines. Endophyte-infected grasses were also tested for their potential to deter wētā from vineyards. Laboratory choice and no-choice experiments demontrated that the loline alkaloids produced by the endophytes in the grasses prevented further feeding by wētā after the initial bite which occurred at the base of their stems. However, this initial bites severed the tillers from the stem and resulted in reduced biomass of endophyte-infected grasses in the no-choice experiment. Results of field experiments from one site also corroborated the potential of these grasses to be used to deter wētā from vineyards. In conclusion, this work proposes a suite of non-pesticidal and sustainable alternatives (shells, under-vine tick beans, endophyte-infected grasses) to mitigate wētā damage in vineyards. These alternatives could either be used alone or together with the current sleeve management approach. Future works could examine combining these strategies into a kind of 'push-pull' wētā management strategy, with 'push' factors comprising endophyte-infected grasses and shells. 'Pull' could comprise strips of non-crop habitats established at the boundaries of vine blocks. Plants in this habitat could consist of tick beans, as well as the shrubs and dicotyledous weeds identified in the insect's diet.

Keywords: Wētā, phylogenetic analysis, morphological keys, DNA barcoding, threat status, conservation, distribution, vineyards, bud damage, budburst, sustainable management, habitat manipulation, diet analysis, metabarcoding, loline alkaloids, endophyte-infected grasses, deterrence, 'push-pull' strategy.

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Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

1.1 Global agriculture

About 7.5 billion people are currently estimated to live on earth and the world's population is projected to reach 9.7 billion by 2050 (DESA, 2015). To meet the demand of feeding an increasing global population, overall food production must increase by about 70% between 2005/7 and 2050. For this increase to be sustainable, most of it must come from existing agricultural land and waste in the current food production system should reduce substantially (FAO, 2009a; Godfray & Garnett, 2014). Over the last decade, modern agricultural production practices have doubled food production to feed mankind using external inputs such as high-yielding cultivars, chemical fertilizers and pesticides, and mechanizations and irrigation (Foley et al., 2005; Smil, 2001). However, that yield increase has remained linear and any further yield increase is anticipated to require increasing the cultivated areas (because yield gains from crop breeding are declining) or through increasing the productivity of the existing agricultural footprint (FAO, 2009b; Godfray et al., 2010; Reid, 1998).

These modern practices have detrimental effects on the environment. For instance, water quality is adversely affected by the increased use of fertilizers (nitrogen and phosphorus). When these are washed, or leached into aquatic systems at high rates, nuisance species dominate. Blue-green algal species can dominate rivers, lakes and streams that receive high rates of P and N loading (Foley et al., 2005; Tilman, 1999a; Tilman et al., 2001). Similarly, irrigation of agricultural lands result in the leaching of agrochemicals into ground and surface water (Hildebrandt, Lacorte, & Barceló, 2009; Tilman, 1999b). Major biodiversity losses are also occurring because of the conversion of forest and other ecosystems to agricultural lands (Rockström, Klum, & Miller, 2015; Tilman et al., 2002). This is undermining important ecosystem functions such as primary production, pest regulation, etc. Consequently, the provision of important ecosystem services such as food, fibre, pollination, and natural pest control are negatively affected (Costanza et al., 1997; Loreau et al., 2001; Swift, Izac, & van Noordwijk, 2004; Tscharntke et al., 2005). High volumes of petro-chemical energy are therefore substituted for key functions in order to achieve the desired efficiencies in the production of specific goods, while maintaining biodiversity below the 'functional threshold' (Swift et al., 2004; Wratten et al., 2012).

These adverse effects of agricultural practices on the earth's environment contribute towards pushing the Earth system outside the stable environmental state that has persisted for over 11,700

years (the Holocene) (Steffen et al., 2015). During that era, environmental changes occurred naturally, and Earth's regulatory capacity maintained conditions that enabled human development. However, the rise of human civilisations and the advent of the industrial revolution has resulted in a new era, known as the Anthropocene, in which human activities are the main drivers of environmental change (Crutzen, 2002; Rockström et al., 2009). These activities could drive most parts of the world into a less hospitable state by affecting certain intrinsic biophysical processes that stabilise the Earth system. These processes include climate change, change in biosphere integrity (i.e., biodiversity loss), stratosphere ozone depletion, ocean acidification, biogeochemical flows (nitrogen and phosphorus), land-use change, freshwater use, atmospheric aerosol loading and the introduction of novel entities such as chemical pollution (Steffen et al., 2015). Of these, climate change, biosphere integrity/biodiversity loss, biogeochemical cycles and land-use change have exceeded thresholds beyond which the Earth's functioning may be substantially altered. For mankind to continue pursuing long-term social and economic development, the Holocene-like condition of the Earth system must be returned (Rockström et al., 2015; Steffen et al., 2015). Achieving this will require the concerted effort of all agricultural production sectors, including viticulture, which relies on high inputs to sustain production.

1.2 Global viticulture

Grapevines belong to the family Vitaceae which comprises 17 genera and about 1000 species that grow in temperate and tropical climates. Although majority of these occur in the tropics or subtropics, it is only one temperate species, *Vitis vinifera* L., which has economic benefits globally (Bouquet, 2011; Keller, 2010b). There are more than 7,000 varieties of this species and they are grown between latitudes of 40° and 50°N in the northern hemisphere and between latitudes of 30° and 45° S in the southern hemisphere (Demir, 2014; OIV, 2016; Wan et al., 2008). Their fruit is one of the most produced fruits in the world, with approximately 75 mt per year. Almost half of grapes produced are vinified, 36% are consumed fresh and 8% are consumed in the form of dried grapes. The rest are used for fruit juice and must production (Keller, 2010b; International Organisation of Vine & Wine, 2016).

As at 2015, the total world area under vine cultivation was 7.534 million ha with Spain (1.021 mha), China (0.82 mha) and France (0.78 mha) having the first, second and third largest areas, respectively. Vineyard areas in China (+34 kha) and New Zealand (+1 kha) increased, while those in the European Union countries decreased slightly (-26 kha) between 2014 and 2015 (International Organisation of Vine & Wine, 2016). The decrease in vineyard areas in Europe is due to an EU programme (which ended in 2011/12) aimed at regulating its wine production potential (International Organisation of Vine & Wine, 2015). Other important wine grape producing countries in decreasing order are Italy,

Turkey, United States of America, Argentina, Portugal, Chile, Romania, Australia, Moldova, South Africa, Brazil and New Zealand (International Organisation of Vine & Wine, 2016). However, in terms of wine production, the top 10 leading countries in decreasing order are Italy, France, Spain, USA, Argentina, Australia, China, Chile, South Africa and Germany (International Organisation of Vine & Wine, 2016).

The production of grapes is of course affected by abiotic and biotic factors. The major abiotic stresses that pose a threat to grape yields are climate (temperature, precipitation, CO_2 concentration etc), drought and salinity. Grapevines grow and produce at temperatures between 12 and 22 °C. Higher temperatures are needed for budburst but temperatures beyond 30°C results in reduced berry size and weight (De Orduna, 2010; Lorenzo, Taboada, Lorenzo, & Ramos, 2013). An increase in CO_2 concentration increases biomass, fruit sugar concentration and decreases acidity (Schultz, 2016), while drought reduces bud fertility and thus affects yield (Guilpart, Metay, & Gary, 2014; Matthews & Anderson, 1989). Salinity results in reduced yield and increases vine mortality (Shani & Ben-Gal, 2005). Thus, climate change and the availability of water for irrigation are expected to greatly impact on vine production (Mozell & Thach, 2014).

Biotic stresses of economic importance to grape production are birds, insect pests and diseases. Pests such as mealybugs (*Pseudococcus calceolariae* Westwood, 1840*, P. longispinus* (Targioni Tozzetti)*, P. viburni* Signoret, 1875), grape phylloxera (*Daktulosphaira vitifoliae* Fitch, 1855), flower thrips (*Thrips obscuratus* Crawford, 1941), light brown apple moth (*Epiphyas postvittana* (Walker, 1863)), European grapevine moth (*Lobesia botrana* (Denis & Schiffermüller, 1775) variegated leafhopper (*Erythroneura variabilis* Beamer, 1929), black vine weevil (*Otiorhynchus sulcatus* Fabricius, 1775) (Daane & Williams, 2003; Gange, Brown, & Sinclair, 1994; King & Buchanan, 1986; Lo, Bell, & Walker, 2009; Schmidt, Roschewitz, Thies, & Tscharntke, 2005; Suckling & Brockerhoff, 2010) attack vines. Diseases of mature vines include *Botrytis cinerea* Persoon, 1794*,* grapevine leafroll disease (caused by a complex of vector-borne virus species in the family Closterpviridae), anthracnose (*Elsinoë ampelina* Shear, 1929), downy mildew (*Plasmopara viticola* (Berlese & De Toni, 1888)) and black foot rot (*Cylindrocarpon* Wollenw., 1913 sp.) (Almeida et al., 2013; Brook, 1992; Elmer & Michailides, 2007). Yield loss due to insect pests range between 12 and 65% depending on the species and vine cultivar, but could be higher when the insects transmit vine disease(s) (Lo & Murrell, 2000). Diseases could also cause as much as 95% yield loss, while reducing grape quality for wine making (Atallah, Gómez, Fuchs, & Martinson, 2011; Calonnec, Cartolaro, Poupot, Dubourdieu, & Darriet, 2004; Munkvold, Duthie, & Marois, 1994).

Management of these insect pests and diseases mainly involves the use of synthetic pesticides (insecticides, fungicides and herbicides) and to a lesser extent on combinations of some cultural

practices (e.g. planting disease-free materials and enhanced vineyard hygiene, especially with regard to infected residues) and biological control (Berndt, Wratten, & Hassan, 2002; Frank, Wratten, Sandhu, & Shrewsbury, 2007). Italy alone has over 200 pesticides registered for use in vineyards and residues have been detected in wines from Italy and other European countries (Baša Česnik, Gregorčič, & Čuš, 2008; Cabras & Conte, 2001; Cunha, Fernandes, Alves, & Oliveira, 2009; Economou, Botitsi, Antoniou, & Tsipi, 2009). These pesticides impact negatively on humans and the environment (van der Werf, 1996). Apart from killing the target organisms, they are toxic to humans, birds, fish, beneficial insects, and non-target plants (Aktar et al., 2009). In humans, the effect is mostly chronic and affected organs are the kidneys and liver (Patil et al., 2003). Insecticides are generally the most acutely toxic class of pesticides, although herbicides can also pose risks to non-target organisms. They contaminate soil, water and other vegetation (Aktar et al., 2009). Hence, the need to adopt alternative approaches for managing existing vine pests and emerging ones.

1.3 New Zealand viticulture

New Zealand has eleven viticultural regions (Imre & Mauk, 2009) with a total vineyard area of 36.192 kha and a mean grape yield of 12.0 t/ha as at 2016 (WineGrowers, 2016). Marlborough is the largest region, accounting for approximately two-thirds of the area, while the remaining areas in decreasing order are in Hawke's Bay, Central Otago, Gisborne, Canterbury/Waipara, Nelson, Wairarapa, Auckland/Northland, and Waikato/Bay of Plenty regions. About 17 varieties of grape are grown but those planted on at least 1 kha of land are Sauvignon Blanc (21.02 kha), Pinot Noir (5.57 kha), Chardonnay (3.2 kha), Pinot Gris 2.46 kha) and Merlot (1.27 kha) (WineGrowers, 2016).

The wine industry is very important for the New Zealand economy, both domestically and in terms of export (WineGrowers, 2016). It creates an estimated 7,700 jobs across grape growing, wine making and cellar door sales as well as contributing significantly to intermediate industries spanning fertilisers to business services, packaging to marketing (NZIER, 2014). Wine was the sixth largest export good with a global value of $1.54 billion in the year to December 2015 (WineGrowers, 2016). Over 68% of these earnings was from exports to U.S.A, UK, Australia, Canada, Netherlands, China, Hong Kong and Germany (WineGrowers, 2016).

In spite of the significant contribution of the industry to the New Zealand economy, winegrowing faces a number of challenges. Some of these include competition from France (particularly in low-priced Vin de Pays products), Chile, South Africa and Bulgaria (Beverland & Bretherton, 1998; Wilson & Goddard, 2004), high excise tax that the government levies on the industry (Edlin, 1997), vineyard variability and its concomitant effect on fruit composition and juice quality (Trought & Bramley, 2011) and yield losses due to insect pests and diseases (WineGrowers, 2016).

1.3.1 Vine diseases and pests in New Zealand

The diseases, grapevine leafroll, eutypa dieback (*Eutypa* Tul. & Tul. spp.), botryosphaeria dieback (*Botryosphaeria* Ces. &De Not. spp.), black foot (*Cylindrocarpon* sp.), botrytis (*Botrytis cinerea*) and powdery mildew (*Erysiphe necator* Schwein., 1834), are economically important New Zealand vineyards (Amponsah, Jones, Ridgway, & Jaspers, 2011; Charles et al., 2006; Charles, Froud, van den Brink, & Allan, 2009; Graham, Johnston, & Weir, 2009; Mugnai, Graniti, & Surico, 1999).

A few insect pest species also damage vines. The nymphs and adults of leafhoppers (*Empoasca fabae* Harris, 1841) feed on vine leaves and shoots in late spring and early summer, while the beetle, *Popillia japonica* Newman, 1841, defoliates vines in mid-late summer. Grape berry moth [*Paralobesia viteana* (Clemens, 1860)] infestation occurs from bloom to fruit maturity (Van Timmeren, Wise, & Isaacs, 2012) and larvae of moths such as the light brown apple moths are important defoliators. Mealybugs (*Pseudococcus longispinus*) are also important vectors of the grapevine leafroll diseases in vineyards (Charles et al., 2006). Recent pests in Marlborough vineyards are grassgrubs (*Costyletra zealandica* (White, 1846)) (González-Chang, 2016) and the ground wētā (*Hemiandrus* sp. 'promontorius' (Johns, 2001)) (Joanne Brady, Constellation Brands NZ, pers. comm., 2014). The latter is thought to cause significant yield losses in the absence of protection.

1.4 Wētā

Wētā is a singular and plural Maori word referring to a group of large (20 – 150 mm), flightless, predominantly nocturnal New Zealand endemic insects in the orthopteran families Rhaphidophoridae and Anostostomatidae ((King, Kennedy, & Wallis, 2003; McIntyre, 2001). There are over 140 species of these insects and they are divided into five groups based on morphological or behavioural features – (i) cave wētā (*Pachyrhamma* Brunner v. Wattenwyl 1888, *Gymnoplectron* Hutton, 1897 and *Turbottoplectron* Salmon, 1948); (ii) giant wētā (*Deinacrida* White, 1842); (iii) tusk wētā (*Anisoura* Ander, 1938, *Motuwētā* Johns, 1997); (iv) tree wētā (*Hemideina* White, 1846); and (v) ground wētā (*Hemiandrus* Ander, 1938) (Cook et al., 2010; Johns, 1997; Macfarlane et al., 2010; Sherley, 1998). All the groups, except cave wētā, belong to the family Anostostomatidae.

Wētā evolved in the absence of mammalian predators and competitors in New Zealand (McIntyre, 2001). However, the predatory activities of mammals [e.g. rats (*Rattus exulans* (Peale, 1848), *R. rattus* (Linnaeus, 1758)), mustelids (*Mustela furo* Linnaeus, 1758, *M. nivalis* Linnaeus, 1766) etc.] introduced by the Polynesians and Europeans in the 10th and 17th Centuries AD, respectively, has resulted in many wētā species becoming rare and threatened. Other threats to these insects include habitat degradation (e.g., de-forestation and fire) and the establishment of exotic plant species (e.g.,

gorse) (Sherley, 1998; Wilmshurst, Anderson, Higham, & Worthy, 2008; Wodzicki & Wright, 1984). Wētā have therefore, constituted 71% of all insects translocated for conservation purpose between 1977 and 2010 in New Zealand (Sherley, Stringer, & Parrish, 2010). Wētā species translocated so far include *Deinacrida rugosa,* Buller, 1871, *D. mahoenui, Motuwētā isolata* Johns, 1997*, Hemideina thoracica* (White, 1842)*, H. crassidens* and *H. ricta* Hutton, 1898 (Watts, Stringer, Sherley, Gibbs, & Green, 2008). A 'wētā recovery plan' was developed to help avert the continued threat to other wētā species (Sherley, 1998). A team of orthopteran specialist periodically review the conservation status of wētā and other insects in New Zealand (Trewick et al., 2012, 2016).

In terms of habitat, wētā mostly live in temperate forest and subalpine environments (Pratt, Morgan-Richards, & Trewick, 2008). Cave wētā are forest species and they occupy dark, damp and cool spaces in crevices or under stones, while some species of giant wētā (e.g., *D. heteracantha, D. mahoenui* Gibbs, 1999) are arboreal and others live in grasslands (e.g., *D. rugosa, D. parva, D. carinata*). Tree wētā live in galleries in trees, but ground and tusk wētā live in burrows in the soil and debris, respectively (Edlin, 1997; Johns, 2001; McIntyre, 2001; Sherley, 1998).

These insects are mostly omnivores, feeding on a range of plant and invertebrate (e.g., flies, moths, beetles etc.) materials. Both native and exotic plant species have been identified in wētā diet because diet studies were mainly conducted after human settlements in New Zealand. Thus, tree wētā (*Hemideina crassidens* (Blanchard, 1851)) is known to ingest leaves, fruits, seeds and flowers of a diverse range of plants (e.g., *Fuchsia excorticata* (Forst. & Forst. f.)*, Pinus radiata* Don*, Pratia angulate* (Forst.) Hook.f., 1844 etc.), in addition to invertebrates (Duthie, Gibbs, & Burns, 2006; Griffin, Morgan-Richards, & Trewick, 2011). The giant wētā, *D. mahoenui* Gibbs, 1999*,* feed on gorse (*Ulex europaeus* Linn.) (Sherley & Hayes, 1993; Stronge, Fordham, & Minot, 1997), while feeding experiments with *D. fallai* Salmon, 1950 and *D. heteracantha* White, 1842 found preference for Lettuce (*Lactuca sativa* Linn.) (Richards, 1973). Tusk wētā feed on leaves (e.g., *Coprosma repens* Rich.*, Pittosporum* Banks ex Sol. spp. etc.) and a wide variety of seeds and fruits (McIntyre, 1998; Winks & Ramsay, 1998). Similarly, cave wētā feed on plant materials such as *Melicytis ramiflorus* Forst. and *Macropiper excelsum* (Forst) Miq. (Richards, 1954). For ground wētā, the plants snowberry (*Gaultheria depressa* Hook), Italian ryegrass (*Lolium multiflorum* Lam.) and fathen (*Chenopodium album* Linn.) have been found in their diet (Burns, 2006; Cary, 1983; Wahid, 1978).

Wētā are generally not recognised as pests in cultivated crops, except a record from an apricot orchard where feeding activity of a ground wētā (*Hemiandrus* sp. 'horomaka' (Johns, 2001)) was reported to result in economic yield losses (Wahid, 1978). However, in the early 2000s, a then-unknown species of ground wētā was found causing significant damage to vine (*Vitis vinifera* Linn.) buds in the Awatere Valley, Marlborough, leading to direct impact on vine yield (Joanne Brady,

Constellation Brands, pers. comm. 2014). Vine buds are compound and contain three distinct growing points, referred to as primary, secondary and tertiary buds. At budburst, it is only the primary one that grows into a shoot. However, if it is damaged, the secondary replaces it. Similarly, the tertiary replaces damaged secondary buds (Keller, 2010b).

Wētā feed on the growing primary bud at budburst or those that grow to replace it (i.e., secondary and tertiary buds) (Joanne Brady Constellation Brands NZ pers. comm, 2014). Damage to the primary buds leads to low yield from clusters growing on shoots arising from the inferior secondary buds, or sometimes no yield if the latter are also destroyed. This is because the tertiary buds that grow to replace the secondary one produce only tendrils. Canes are not produced for the next season if the whole compound bud is destroyed (Creasy & Creasy, 2009; Joanne Brady Constellation Brands NZ pers. comm, 2014). Grape growers are not interested in registering an insecticide to control this wētā because it is endemic to New Zealand, culturally significant to the Maori (i.e., of taonga status) and its threat status may worsen if those in vineyards are killed. Also, the wētā problem is restricted to the Awatere Valley, so no company will register a pesticide for it.

1.5 Current wētā management and research approach

Damage to date is managed by tying polythene sleeves (Fig. 1.1) around vine trunks. These are slippery and make it difficult for wētā to climb the vine trunks. This method is thought to be effective in stopping damage. However, the life span of the sleeve is not known and they litter the environment when they are removed by grazing sheep or machinery in vineyards and blown off by the strong winds in the Awatere Valley. This management option is also labour intensive as these sleeves have to be tied around the trunks of individual vines. This increases labour cost and the sleeves often need to be repaired/replaced, leading to further costs. The average cost of tying the sleeves for a hectare is about $415.00, but the repair/replacement cost depends on the number of vines that have their sleeves requiring repair. Furthermore, tying sleeves or repairing/replacing them compete for labour with other important vineyard cultural practices such as vine pruning and training, pest and disease monitoring, canopy management irrigation etc (Joanne Brady, Constellation Brands NZ, pers. comm., 2014).

Figure 1.1 Plastic sleeve on a vine trunk

There is therefore the need to develop an efficient, environmentally safe and sustainable wētā management technique with lower labour and environmental costs to complement and/or replace the existing method. The ideal technique should be able to conserve the wētā as well as significantly reduce their damage to vines, i.e., deter and not kill them.

In eastern Africa, stemborers and striga weed, *Striga hermonthica* (Delile) Benth., damage in maize was successfully controlled by developing a 'push-pull' management technology for these pests. The 'push-pull' pest management strategy basically combines behaviour-modifying stimuli to manipulate the distribution and abundance of pest and their natural enemies for effective pest management in farming systems. This strategy works through the integration of stimuli that repel or deter, or that mask host apparency and thus, 'pushes' pests away from the main crop. The pests are then simultaneously attracted (pulled) towards a border crop from where they are subsequently concentrated, facilitating their elimination by pesticides or natural enemies. Generally, the components of push-pull strategy are nontoxic and reduce the use of insecticides (Cook et al., 2007; Reddy 2016). For the stemborers and striga weed management mentioned earlier, this involved intercropping maize with desmodium (*Desmodium* Desv. spp.) or molasses grass (*Melinise minutiflora* P. Beauv.) (which repels stemborer moths) and planting Nappier grass (*Pennisetum purpureum* Schumach.) or Sudan grass (*Sorghum vulgare sudanense* (Piper)) as a border crop to attract them. Desmodium also suppressed the growth of the parasitic striga weed. Molasses and Sudan grasses increased parasitism of the stemborer by its natural enemies, while Nappier grass produced a gummy substance that restricted larval development, causing a few to survive (Cook, Khan, & Pickett, 2007; Khan, Midega, Pittchar, Pickett, & Bruce, 2011; Khan, Midega, Amudavi, Hassanali, & Pickett, 2008; Khan, Midega, Bruce, Hooper, & Pickett, 2010).

This concept has since been extended for controlling insect pests in crops such as oilseed rape (*Brassica napus* Linn.), cotton (*Gossypium hirsattum* Linn.), potato (*Solanum tuberosum* Linn.*)*, onion (*Alium cepa* Linn.) etc. The stimuli involved in repelling or attracting pests were also identified and are commercially available and included in a 'push-pull' system to increase efficiency (Cook, Khan, & Pickett, 2006; Cook et al., 2007; Hassanali, Herren, Khan, Pickett, & Woodcock, 2008). This approach could therefore be exploited for wētā management by identifying potential 'push' and 'pull' factors for this pest in vineyards.

1.6 General objective

This PhD work aimed at developing an ecologically-based integrated management strategy for wētā in vineyards based on an in depth understanding of the species present, their ecology and habitat.

1.6.1 Specific objectives and hypotheses

The specific objectives of this study and the hypotheses tested under each were;

1. Identify the wētā species associated with vine damage as well as study its density, distribution and aspects of its biology relevant to mitigating its damage to vines

    Hypothesis 1: H_0 = All the wētā damaging vines in the Awatere Valley, Marlborough are of the same species

    Hypothesis 2: H_0 = The densities of this wētā in vine and non-vine habitats are the same

    Hypothesis 3: H_0 = The density and distribution of wētā in different vineyards locations (edge, centre, under vines, inter-rows) are similar

    Hypothesis 4: H_0 = Edaphic factors do not have an effect on the density and distribution of wētā in vineyards

    Hypothesis 5: H_0 = Life history traits such as oviposition and sex ratios are not influenced by seasons in a year

2. Use information on the range of plant species in the diet of this wētā to determine the effect of habitat modification on its pest status

    Hypothesis 1: H_0 = Wētā are pest because of the reduced plant diversity in vineyards

3. Test the efficacy of habitat modification strategies at reducing wētā damage to vines and the effect of these strategies on grape quality

   Hypothesis 1: H_0 = Mussel shells or straw mulch will serve as a physical barrier and prevent wētā emerging from their burrows to feed on vine buds at budburst

   Hypothesis 2: H_0 = Sowing tick beans (*Vicia faba* Linn. var. *minor* (Fab.)) in vineyards as alternative food for wētā will reduce vine bud damage at budburst

   Hypothesis 3: H_0 = Tick beans sown in the inter-rows will be as effective as those under vines in reducing wētā damage to vines

4. Identify plant species that can be used to 'push' wētā out of vineyards

   Hypothesis 1: H_0 = Endophyte-infected grasses can deter feeding by wētā

   Hypothesis 2: H_0 = Endophyte-infected grasses planted as inter-row vegetation in vineyards will 'push' wētā out of vineyards because of limited availability of plant food, thereby reducing vine bud damage

1.7 Thesis structure

The outline of this thesis is shown in Table 1.1.

Table 1.1 Thesis outline

Chapter/ Title	Purpose
Abstract	Summarises the research conducted and key findings
1 General introduction	Gives a background to this PhD work. It examines global agriculture and how it is currently feeding the world's population by relying on petro-chemicals, as well as the consequences of such practices with projected human population increases. The contribution of viticulture to these negative consequences of modern agriculture are discussed. The economic importance of viticulture in New Zealand and the challenges it faces are discussed, followed by an introduction to the new pest, wētā, in Marlborough vineyards. The specific objectives that will feed into the general objective of managing this new pest are presented along with the hypotheses for each.
2 – 5 Research chapters	All the research chapters have the structure: Abstract Introduction – this contains detailed background to the research in that chapter and discusses previous studies relevant to the topic. It ends by stating the objectives and hypotheses being tested.

	Materials and methods – this describes in detail the procedures followed in conducting the research. It also describes how data were collected and analysed. Results – the findings of the study are presented here. Discussion – the findings are discussed and compared with existing literature.
6 Overall discussion and conclusions	This chapter broadly discusses all the experiments conducted and their implications. It summaries the findings and highlights future work that can be done.
References	A detailed list of all the sources from which knowledge and other significant information was acquired.

Chapter 2

Identification, density, distribution and biology of ground wētā

A version of this chapter was published in July 2016: Nboyine JA, Boyer S, Saville D, Smith MJ, Wratten SD (2016). Ground wētā in vines of the Awatere Valley, Marlborough: biology, density and distribution. *New Zealand Journal of Zoology*, 1-15. DOI: 10.1080/03014223.2016.1193548

2.1 Abstract

Ground wētā comprise approximately 40 species of insects and they all belong to the genus *Hemiandrus*. Some of these species are threatened but others are not. A population of wētā from this genus has become a pest in vineyards in the Awatere Valley, Marlborough. This work aimed at identifying the species damaging vines and studying its biology, density and distribution in and around vineyards. DNA barcoding and morphological keys were used to confirm the identity of wētā randomly sampled from six vineyard blocks in this valley. Wētā density was assessed in vineyards, paddocks and shrublands in this valley. Soil moisture, penetration resistance, pH and organic matter were recorded at locations with and without wētā. The wētā damaging vines was identified as *Hemiandrus* sp. 'promontorius'. This species is not threatened, but has a restricted habitat range. Its density in vineyards was significantly higher than that in either paddocks or shrub habitats. In vineyards, the density was significantly higher under-vines than in the inter-rows. Higher numbers of this wētā were found in moist soils that required lower force to burrow. Females laid a mean of 55 eggs between March and April, and these eggs hatched in September. These findings suggest that current viticultural practices do not threaten wētā inhabiting vineyards. Hence, vineyard managers and conservation workers should work together to continue protecting this endemic insect.

Key words: New Zealand, *Hemiandrus* sp. 'promontorius', ground wētā, Awatere Valley, density, vineyards, reproduction

2.2 Introduction

Wētā in the family Anostostomatidae comprise approximately 60 species belonging to the five genera *Hemideina, Deinacrida, Anisoura*, *Motuwētā* and *Hemiandrus* (ground wētā) (Macfarlane et al., 2010; Taylor-Smith, Trewick, & Morgan-Richards, 2016). Of these, the latter is the most speciose and in need of most taxonomic and ecological work (Johns, 2001; Smith, Morgan-Richards, & Trewick, 2013; Taylor-Smith et al., 2016). This is because only 14 of the approximately 40 species in the genus *Hemiandrus* are formally described to date. The rest are referred to by tag names (Jewell,

2007; Johns, 1997, 2001; Smith et al., 2013; Taylor-Smith et al., 2016). This makes them the least well-characterised wētā group in New Zealand. The 14-described ground wētā and their authors are;

Hemiandrus maculifrons (Walker, 1869)

H. pallitarsis (Walker, 1869)

H. focalis (Hutton, 1897)

H. bilobatus Ander, 1938

H. fiordensis (Salmon, 1950)

H. nitawētā Jewell, 2007

H. subantarticus (Salmon, 1950)

H. superba Jewell, 2007

H. lanceolatus (Walker, 1869)

H. maia Taylor-Smith, 2013

H. electra Taylor-Smith, 2013

H. luna Taylor-Smith, 2016

H. brucei Taylor-Smith, 2016

H. nox Taylor-Smith, 2016

Below are the tag names of the undescribed species to date (Johns, 2001; Trewick et al., 2016):

Hemiandrus "onokis"

H. "disparalis"

H. "dodsons"

H. "elegans"

H. "porters"

H. "furoviarus"

H. "hapuku"

H. "horomaka"

H. "kapiti"

H. "madisylvestris"

H. "mtgeorge"

H. "nokomai"

H. "promontorius"

H. "pureora1"

H. "pureora2"

H. "redhills"

H. "richmond"

H. "saxatilis"

H. "staveley"

H. "timaru"

H. "turgidulus"

H. "waimakariri"

H. "vicinus"

H. "otautau"

H. "otekauri"

H. "Cromwell"

H. "tapuae-O-uenuku"

H. "small lake"

H. "sp. near focalis"

The presence of many tag names is because the identifications of ground wētā have generally been challenging, with some poor descriptions, confusions in early nomenclature and a history of misidentified specimens (Johns, 2001). For instance, in the past, this group was thought to comprise the two genera, *Zealandosandrus* Salmon 1950 and *Hemiandrus* Ander 1838. This classification was based on the length of their ovipositor. Thus, *Zealandosandrus* referred to wētā with long ovipositors, while *Hemiandrus* were those with short ovipositors and modified 6th abdominal sternites of females. Later, they were all placed in the genus *Hemiandrus,* a decision supported by phylogenetic analysis (Johns, 1997; Pratt et al., 2008; Salmon, 1956).

Ground wētā are all nocturnal and each species is found at specific locations in the North and South Islands of New Zealand, although some (e.g., *H. maculifrons* (Walker, 1869), *H. luna* Taylor-Smith 2016, *H. brucei* Taylor-Smith 2016; *H. nox* Taylor-Smith 2016) occur on both islands (Chappell et al., 2012; Pratt et al., 2008; Taylor-Smith et al., 2016). The habitat preference of some of these ground wētā is partially separated by elevation. For example, *H. pallitarsis* Walker, 1869 is found at lower altitudes than *H. maculifrons* (Walker, 1869) (Chappell et al., 2015). Actual data about the biology, density and distribution of most species in this group is limited because of their subterranean and nocturnal habit (Johns, 2001). This has resulted in frequent changes in their conservation status. For instance, *H. nitawētā* and *H. superbus* which were listed in 2012 as not threatened are now listed as Naturally Uncommon because they are known only from Sinbad Gully, Fiordland, while the status of *H.* sp. 'Kapiti' and *H. electra* have changed to Naturally Uncommon and Not Threatened respectively, because more is known about their distribution (Trewick et al., 2016; Trewick et al., 2012). Increased knowledge of the distribution of this group of wētā and an understanding of factors potentially affecting their density and distribution within a habitat is vital for protecting those threatened. This will also help protect species inhabiting agricultural areas, even if they are not threatened, and thus prevent them from assuming a 'threatened' status.

This chapter uses DNA barcoding and morphological tools to establish the identity of wētā damaging vines as well as studying the density, distribution and aspects of the biology of this wētā in the Awatere Valley, Marlborough.

This information is considered basic for designing strategies to mitigate damage by wētā in the affected vineyards. Knowing the exact species causing damage and therefore, its conservation status

will inform the type of management strategy to develop. Baseline data on the numbers of this insect currently inhabiting vineyards, and their biology will contribute towards measuring the negative effect(s) of the proposed conservation management strategies on this insect. This will also ensure that declines in wētā numbers after adopting any management method can be identified and potentially ameliorated.

2.3 Materials and methods

2.3.1 Study sites and period

The study was conducted in the Awatere Valley, which is south of Blenheim, south-east of the Wairau Plains and north of Cape Campbell, Marlborough. The distance from Cape Campbell to the valley is 53 km. The study took place from 19 May 2014 to 6 November 2015.

This valley has a more extreme climate than most of Marlborough. The total annual rainfall is 450 – 1000 mm and its mean minimum and maximum monthly air temperatures are 0.6 and 24.2 °C, respectively. It also has a mean monthly maximum wind speed of 78.3 km/hr (http://www.mrc.org.nz/category/weather-data/awatere-valley-dashwood-weather-data/. Accessed 20 January, 2016).

The grape variety in the vineyards used for the study was Sauvignon Blanc although wētā can also be found in vine blocks containing other varieties such as Pinot Noir.

2.3.2 Identification of wētā

2.3.2.1 Wētā sampling

Wētā were sampled randomly from six vineyard blocks located at Caseys Road, The Favourite and Castle Cliffs in the Awatere Valley (Table 1). In all, 34 individual specimens were used for this work.

Table 2.1 Names and locations of vineyard blocks used to monitor seasonal wētā densities.

Location	Name of vineyard blocks	Area of block (Ha)	GPS Coordinates	Elevation (m.a.s.l.)
Castle Cliffs	O- Block	4.61	-41.6103 °S, 174.1276 °E	21
Castle Cliffs	D- Block	37.88	-41.6075 °S, 174.1328 °E	28
Castle Cliffs	H- Block	2.98	-41.6131 °S, 174.1359 °E	8
The Favourite	L- Block	16.88	-41.6198 °S, 174.1071 °E	46
The Favourite	N- Block	44.41	-41.6260 °S, 174.1105 °E	43
Caseys Road	H- Block	11.98	-41.6880 °S, 174.120 °E	22

2.3.2.2 DNA extraction

The tibia of the hind leg of each of the 34 wētā was used for DNA extraction. A Zymo Research (ZR) Tissue & Insect DNA MicroPrep ™ kit was used for the extraction following the manufacturer's instructions with slight modification. Briefly, the hind tibia of each insect was cut off with a scalpel and placed in a 0.5 ml tube followed by freeze drying in liquid nitrogen. These specimens were then crushed inside the tubes with a pestle. The scalpel was sterilised by passing it successively through three 50 ml tubes two-thirds filled with bleach, ethanol and deionised water respectively, while pestles were used once for each sample after which they were sterilised overnight in bleach.

To each of the tubes containing the crushed, freeze dried tissues (< 10 mg), 750 µl of lysis solution was added. The tubes were warmed on a hot plate for 10 minutes at 25 °C. This was followed by centrifuging the tubes at 10, 000 × g for 1 minute. The supernatant (400 µl) was transferred to a Zymo-Spin ™ IV Spin Filter in a collection tube and centrifuged at 7000 × g for a minute. Genomic lysis buffer (1,200 µl) was added to the filtrate in the collection tube, after which 1,600 µl (in two batches of 800 µl) of the mixture was transferred to Zymo-Spin ™ IC column in a collection tube

followed by centrifuging at 10, 000 × g for a minute. The collection tubes were emptied after each transfer. The Zymo-Spin ™ IC column was placed in a new collection tube followed by adding 200 µl of DNA Pre-Wash Buffer and centrifuging at 10, 000 × g for 1 minute. Another 500 µl g-DNA Wash Buffer was added to the Zymo-Spin ™ IC columns and they were centrifuged for 1 minute at 10, 000 × g. The columns were each transferred into a clean 1.5 ml microcentrifuge tube and 20 µl DNA Elution Buffer was added directly into their column matrix. They were then centrifuged at 10, 000 × g for 30 seconds to elute the DNA.

2.3.2.3 Polymerase chain reaction (PCR) and electrophoresis

PCR was performed using the universal primer pair HCO 2198 and LCO 1490 that target the COI gene region. The amplification was performed in 10 µl reaction mixtures containing 1.5 µl DNA extract, 1.3 µl water, 5 µl GoTaq® Green 2 ×, 0.5 µl bovine serum albumin (BSA, 10 mg/ml), 0.5 µl $MgCl_2$ (25 mM) and 0.8 µl each of the forward and reverse primers (10 µM). The protocol for the thermocycling was: 94 °C for 5 min, 38 cycles of 94 °C for 45 s, 48 °C for 45 s and 72 °C for 1.20 min, and a final elongation at 72.0 °C for 7 min. Controls comprising DNA of a beetle (positive) and PCR grade water (negative) as templates were included in the PCRs to check for the success of amplification and DNA contaminations, respectively. The PCR products underwent electrophoresis using a loading buffer in an Agarose & Sybrsafe gel 75 v for 45 min. The gels were viewed under UV-light using an Invitrogen Safe Imager ™ for the presence of bands of expected size.

2.3.2.4 Cleaning of PCR products and sequencing PCR

PCR products that showed bands of expected size were cleaned using an Agencourt® AMPure® XP PCR purification kit. Briefly, this involved pipette mixing 10 µl of the PCR product with 18 µl AMPure® XP 10 times. The mixed samples were incubated for five minutes at room temperature (20 °C). The reaction plate was placed onto an Agencourt SPRIPlate 96 Super Magnet Plate for two minutes to separate beads from the solution. The resulting clear solution was aspirated and discarded without removing the reaction plate from the magnetic plate. To each well of the reaction plate, 200 µl of 70% ethanol was added followed by incubating for 30 s at room temperature on the magnetic plate. The ethanol was aspirated and discarded, and the whole process of washing with ethanol repeated twice. Off the magnetic plate, 40 µl of PCR grade water was added to each well of the reaction plate and pipette mixed 10 times. The reaction plate was then placed on the magnetic plate for a minute to separate beads from the reaction mixture. The eluate (cleaned PCR products) was then transferred onto a new plate.

After purification, sequencing PCR was performed in 10 µl reaction mixtures comprising 0.5 µl cleaned PCR product, 6 µl water, 2 µl 5 x buffer, 0.5 µl BigDye ™ Terminator chemistry and 1 µl LCO 1490. The thermocycling protocol was: 96 °C for 1 min, 25 cycles of 96.0 °C for 10 s, 50 °C for 5 s and

60 °C for 4 minutes, ending with an elongation at 60 °C for 1 min. Samples were then sequenced on an Applied Biosystems 3130 xl Genetic Analyzer.

2.3.3 Analysis of genetic data

The resulting sequences were analysed using MEGA v. 7 software. Individual sequences were inspected for unexpected insertions and deletions of amino acids in comparison to the chromatograms. Sequences obtained from the reverse primer were reversed and converted to their complementary nucleotides and aligned with the corresponding sequences for the same specimen, using the forward primers, thus lengthening the fragment. Overlapping fragments from individual specimens were then aligned to assess their similarity to each other.

The Basic Local Alignment Search Tool (BLAST) was used to match nucleotide sequences with the most similar ones that have been registered on GenBank. For the final analysis, sequences from 12 specimens from this study were used together with another eight sequences from related specimens on GenBank, *Hemiandrus* 'promontorius' (GenBank accession numbers: JF895564.1, EU676789.1, EU676777.1), *H. bilobatus* (JF895563.1, JF895562.1, EU676794.1), and *H. pallitarsis* (JF895608.1, JF895606.1 JF895605.1). MEGA v.7 was then used to construct a phylogenetic tree and evolutionary divergence table using the neighbour-joining method. Maximum Composite Likelihood method (Tamura, Nei, & Kumar, 2004)was used to compute evolutionary distances.

Taxonomic data keys (Johns, 2001) were used to confirm the identity of the species when sequencing results were inconclusive.

2.3.4 Distribution and density *H*. sp. 'promontorius'

2.3.4.1 Density of *H*. sp. 'promontorius' in different habitats

Densities of *H*. sp. 'promontorius' in three habitat types (vineyards, paddocks and shrublands) commonly found in the Awatere Valley were estimated in January (summer) and November (spring) 2015, by searching for this insect and its burrows in each habitat. The shrublands were dominated by gorse (*Ulex europaeus* Linn.), gum tree (*Eucalyptus* sp. L'Hèr.)), willow (*Salix* sp. Linn.), ngaio (*Myoporum laetum* Forst.), matagouri (*Discaria toumatou* Raoul) and cabbage tree (*Cordyline australis* (Forst.)). Five different locations (Castle Cliffs, Barker's Marque Wines, Pernod Ricard NZ, Heard Vineyard and Villa Maria), which were at least 3 km apart, were used. At each of these locations, a single habitat of paddock, shrubland and vineyard were sampled. Thus, a total of 15 sampling sites (i.e. 5 locations × 3 habitats) were sampled for this insect during the study.

Within each of the 15 sites, five 100 m^2 plots were randomly demarcated and carefully searched for wētā and their burrows (Fig. 2.1). The presence of the latter was determined by scraping off the top

5 mm soil layer. Grassy/weedy plots within each habitat were searched by clearing the grasses and/or weeds before scraping off the topsoil layer to a depth of 5 mm to expose all burrows present. Three burrows were randomly selected and dug within each plot and the numbers of wētā present were counted.

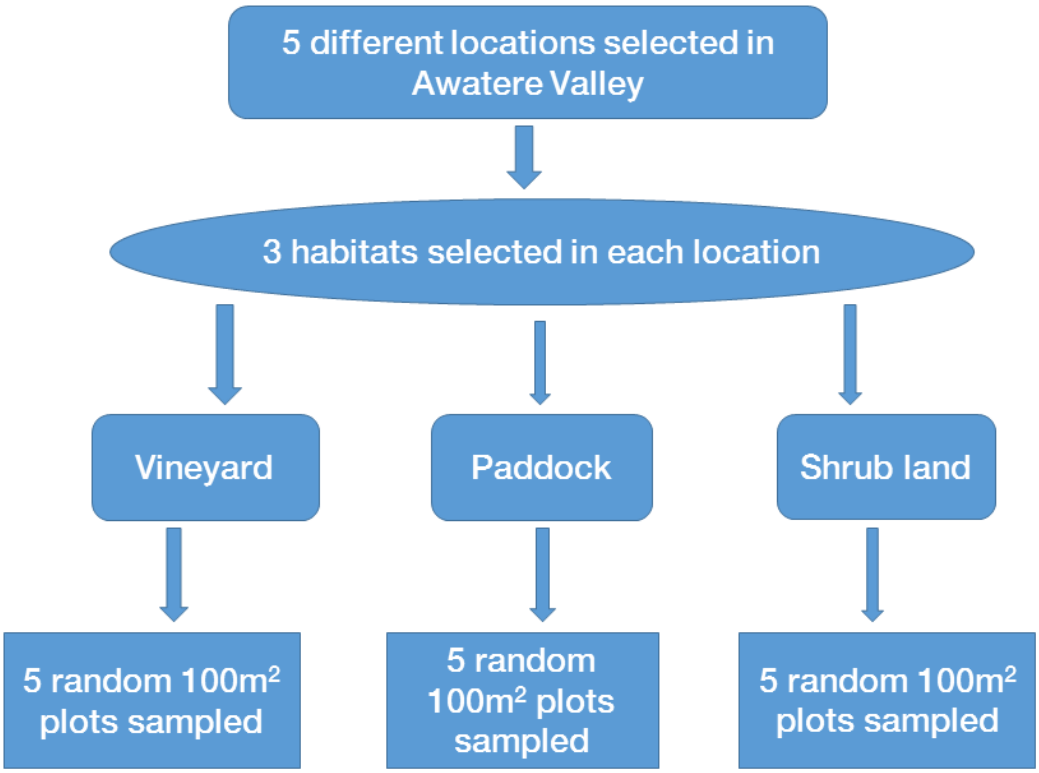


Figure 2.1 Sequence of wētā sampling in each type of habitat

Wētā counts in each habitat were converted into density (i.e. number of wētā/ m^2). These data were subjected to randomised complete block Analysis of Variance (ANOVA) with location as the blocking factor and habitat as the treatment factors.

2.3.4.2 Distribution and seasonal pattern in vineyards

A stratified sampling method was used to assess the distribution of wētā in vineyards and their density fluctuations in different seasons (Fig. 2.2). Six vineyard blocks located at Caseys Road, The Favourite and Castle Cliffs (see Table 1 for vineyard details) were sampled in May (autumn), July (winter), and October (spring) of 2014 and January 2015 (summer).

Three vine rows and their adjacent inter-rows were randomly selected for sampling on each of the dates and for each of the six blocks (Fig. 2.3). Vines were planted at inter-vine and inter-row spacing of 1.8 m and 2.4 m, respectively. Within each row, bays were 7.2 m long and comprised four vines.

The selected rows, which ran south to north in all vineyard blocks, were divided into 'edge' and 'centre'. The 'edge' consisted of the first three complete bays and it was sampled by digging a 250 x 250 x 300 mm (length x breadth x depth) hole in the middle of each bay and its corresponding point in the middle of the inter-row on the east side of the sampled row.

The 'centres' consisted of the area between bays 6 and 19 in the row. The under-vines and inter-rows of five randomly chosen bays in this area were sampled by digging as described for the edge. A total of 48 samples (i.e. 3 rows × 16 samples/row) were taken per vineyard in four seasons and sampling was conducted from the south to north end.

All excavated holes were carefully searched for the presence of wētā and their eggs. This information was considered fundamental to the understanding of biology of this pest. Adults were sexed using the general descriptions for male and female ground wētā (Van Wyngaarden, 1995). The number of females brooding eggs was recorded and numbers of eggs /female were counted. The females and eggs were returned to the soil afterwards.

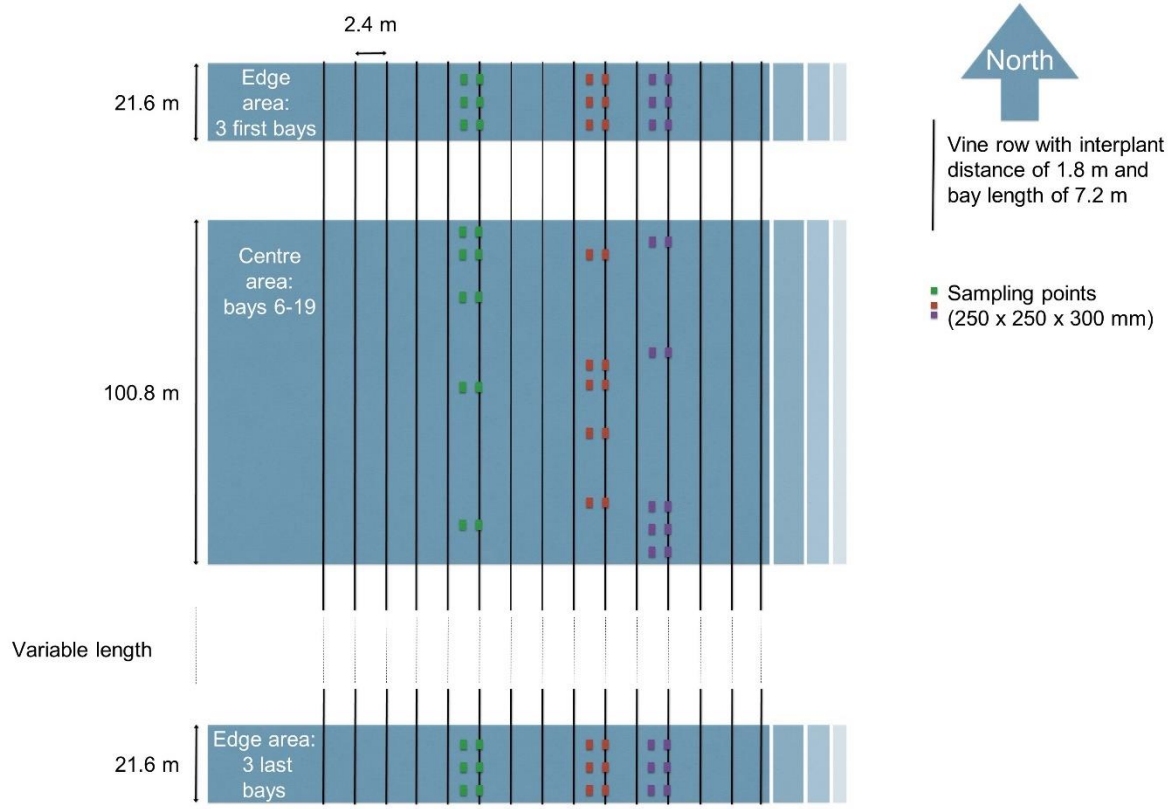


Figure 2.2 Example of sampling design on one date at one vine block (48 samples)

Figure 2.3 Under-vine and inter-rows locations sampled in a vine block

Data from each season were converted into mean number of wētā/m^2 for under-vines, inter-rows, edge and centre and overall weighted means were calculated. This was followed by computing the 95% confidence intervals for the different dates and sampling positions in vineyard blocks.

Statistical differences between the numbers of male and female wētā were determined by testing the null hypothesis, H0: the proportion of males = 0.5, using the Minitab® 17 statistical programme. Chi-squared tests were performed to determine if the sex ratio and proportion of brooding females changed with time.

2.3.5 Measurement of soil properties

The soil variables measured were considered appropriate to the study locations, which were all close together in the Awatere Valley, with negligible variation in slope and altitude. Therefore, the latter variables were not considered.

2.3.5.1 Volumetric soil moisture

This was measured in each of the vineyard blocks using a Delmhorst KS-D1 Digital Soil Moisture Tester. The meter was calibrated to measure moisture up to 200 mm below the soil surface. The probe of the moisture meter was inserted next to each hole dug to a depth of 50 mm.

2.3.5.2 Soil pH

The soil pH in the vineyard blocks was measured by collecting soil to a depth of 200 mm. The soil was placed in zipped plastic bags and immediately frozen to stop any chemical or biological processes that could alter the pH. Forty-eight soil samples were collected from each of the six vineyard blocks.

In the laboratory, soil sub-samples (>10 g) were taken from the contents of each bag and emptied into individually labelled plastic trays; they were dried in an oven at 25 °C for 48 h. The soils were then ground and 10 g of each sample were transferred to clean plastic vials. Deionised water (25 ml) was added to the contents of each vial, after which they were left on the laboratory bench for 24 h. The pH of each sub-sample was measured with an Orion™ Star A211 pH, mV, ORP and temperature bench-top meter.

2.3.5.3 Soil organic matter

After pH measurements were taken, the remaining soil was used to determine organic matter content. This was done by weighing 10–20 g of those soils into labelled crucibles and then drying them in an oven at 105 °C for 24 h. The dried soils were cooled in a desiccator for 30 minutes before it was weighed and then burned in a furnace at 500 °C for 5 h. Organic matter content was computed based on the weight loss after burning (Blakemore, 1987).

2.3.5.4 Resistance to soil penetration

This was measured at each of the points sampled in the vineyard blocks using the 3cm^3 cone of the static Eijkelk® cone penetrometer. A total of 48 readings were recorded/ vineyard block/ season. A constant penetration velocity of approximately 30 mm/s (ASAE, 1998) was used to drive the cone into the soil to a depth of 15 cm. The force required to push it to this depth was recorded in KNewton.

2.3.6 Field data analysis

The statistical software GENSTAT® Version 16.0 was used to perform regressions of relationships between the density of wētā and soil properties.

2.4 Results

2.4.1 Genetic and morphological identification of wētā

Of the 34 specimens from which DNA was extracted, only 12 good-quality chromatograms were obtained that could be used for subsequent analyses. These sequences ranged from 550 – 680 base pairs. BLAST searches matched them closely to only one of the three Genbank sequences submitted as *Hemiandrus* sp. 'promontorius' GW 193 (COI) JF895564.1 with maximum identifications ranging from 95 and 100%. The latter (i.e., JF895564.1) was submitted by Chappell et al. (2012). A

phylogenetic analysis of the sequences from this work confirmed that they were all the same species. All 12 sequences from this work and JF895564.1 from Chappell et al. (2012) clustered separately in a neighbour-joining tree. Similarly, *H. bilobatus* and *H. pallitarsis* (sequences submitted by Chappell et al 2012 Chappell et al. (2012)) each formed different clusters (Fig. 2.4).

The intraspecific divergences between the vineyard specimens ranged between 0.0 and 1.6%. There was also a 0.2 – 1.4% divergence between the specimens from this work and JF895564.1, indicating that they were likely to be the same species (Hebert, Ratnasingham, & de Waard, 2003) (Table 2.2).

Interspecific divergences between sequences from this work's specimens and the two closely related ground wētā, *H. bilobatus* (JF895563.1, JF895562.1) and *H. pallitarsis*, (JF895608.1, JF895606.1 JF895605.1) were > 5.1% and 21%, respectively (Table 2.2). This creates a high barcode gap (i.e., ratio of inter- to intra- specific divergences) between the specimens from vineyards and *H. pallitarsis* from Genbank, but not with *H. bilobatus* (because it is less than 10%).

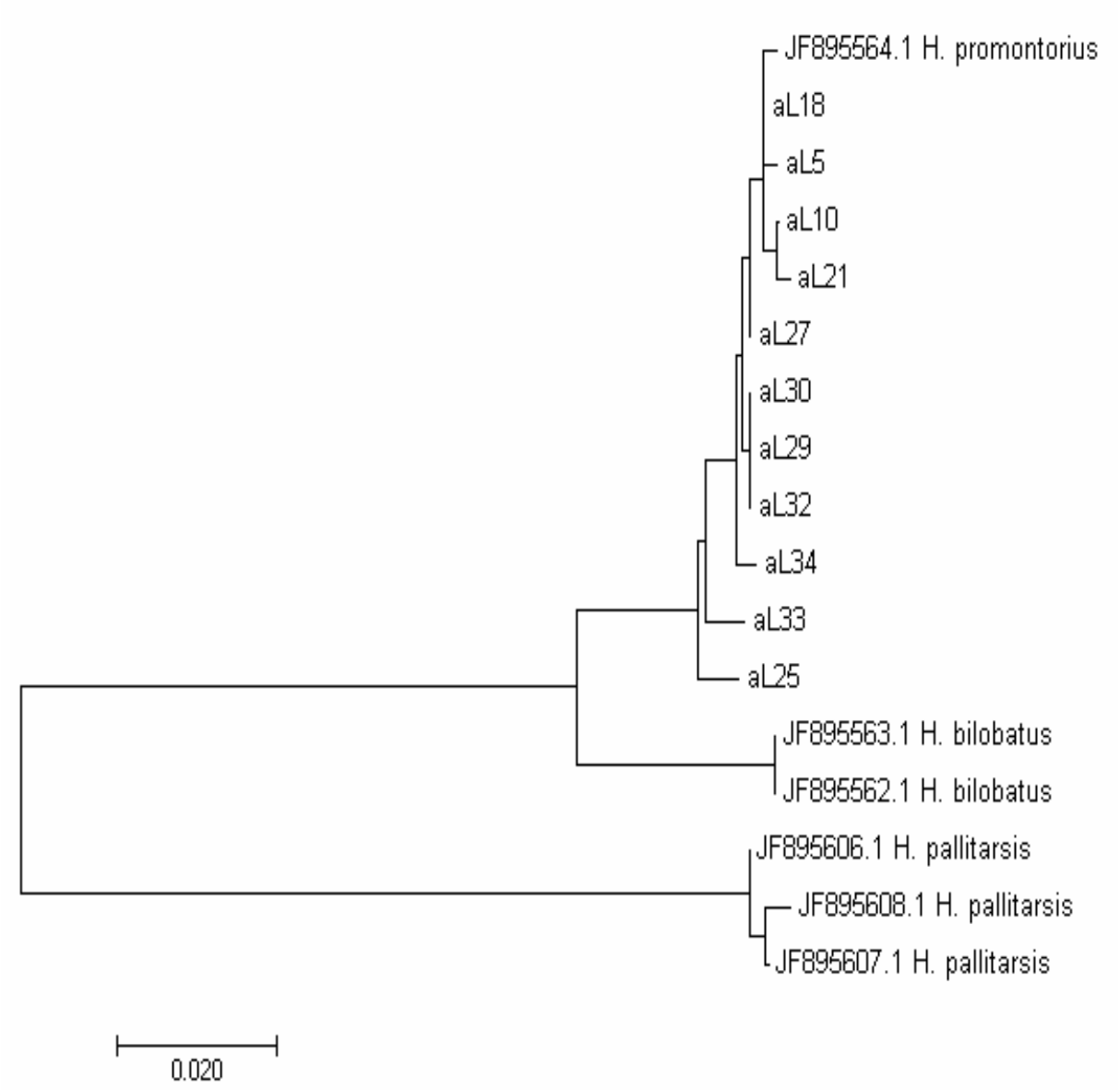


Figure 2.4 Molecular phylogenetic analysis by the Maximum Likelihood method.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 0.23378714 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura & Nei, 1993) and are in the units of the number of base substitutions per site. The analysis involved 17 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 563 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar, Stecher, & Tamura, 2016).

Table 2.2. Estimates of evolutionary divergence between sequences

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	JF 895564.1 *H.* 'promontorius'																
2	JF895563.1 *H. bilobatus*	0.047															
3	JF895562.1 *H. bilobatus*	0.047	0.000														
4	JF895608.1 *H. pallitarsis*	0.190	0.190	0.190													
5	JF895607.1 *H. pallitarsis*	0.188	0.187	0.187	0.004												
6	JF895606.1 *H. pallitarsis*	0.185	0.184	0.184	0.005	0.002											
7	aL5	0.004	0.047	0.047	0.188	0.185	0.182										
8	aL10	0.004	0.051	0.051	0.193	0.190	0.188	0.004									
9	aL21	0.005	0.053	0.053	0.196	0.193	0.190	0.005	0.002								
10	aL25	0.016	0.047	0.047	0.183	0.180	0.178	0.016	0.016	0.018							
11	aL27	0.004	0.047	0.047	0.188	0.185	0.182	0.004	0.004	0.005	0.013						
12	aL30	0.005	0.045	0.045	0.185	0.182	0.180	0.005	0.005	0.007	0.011	0.002					
13	aL29	0.005	0.045	0.045	0.185	0.182	0.180	0.005	0.005	0.007	0.011	0.002	0.000				
14	aL32	0.005	0.045	0.045	0.185	0.182	0.180	0.005	0.005	0.007	0.011	0.002	0.000	0.000			
15	aL34	0.007	0.047	0.047	0.188	0.185	0.182	0.007	0.007	0.005	0.013	0.004	0.005	0.005	0.005		
16	aL33	0.014	0.049	0.049	0.186	0.184	0.181	0.014	0.014	0.016	0.009	0.011	0.009	0.009	0.009	0.011	
17	aL18	0.002	0.049	0.049	0.190	0.188	0.185	0.002	0.002	0.004	0.014	0.002	0.004	0.004	0.004	0.005	0.013

The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Maximum Composite Likelihood model (Tamura et al., 2004). The analysis involved 17 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 563 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

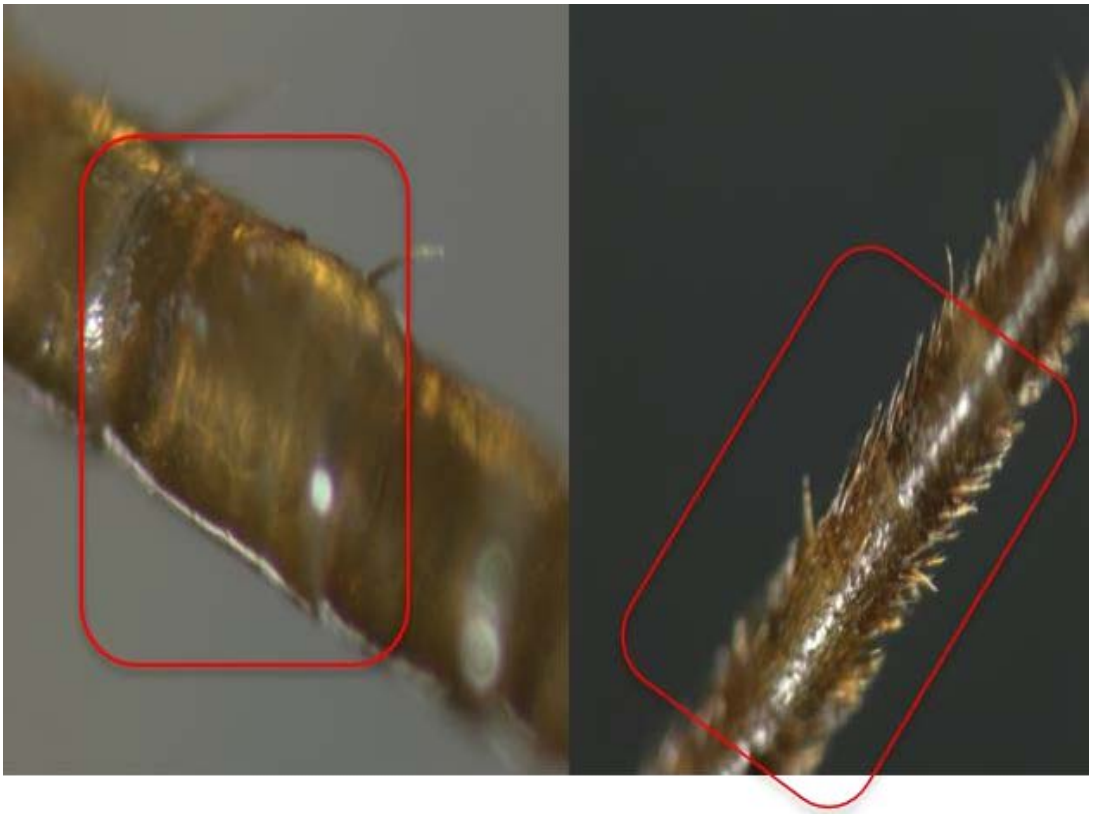
All specimens were identified using taxonomic keys developed by Johns (2001). Based on these keys, the major distinguishing features of *Hemiandrus* sp. 'promontorius' observed on all specimens were;

1. The presence of 10 basal glabrous segments of the antennomeres (Fig. 2.5A)
2. The number of spines close to the midpoint of the front tibia were two on each side (Fig. 2.5B)
3. The middle tibia had two prolateral and four retro-lateral spines
4. The prolateral and retrolateral spines on the midtibia of all specimen were in pairs

The specimens were also sent to a wētā taxonomist (Peter M. Johns) at the Canterbury Museum, Christchurch for morphological confirmation of the species' identity.

The results from both the genetic and morphological identifications clearly showed that all specimens were *Hemiandrus* sp. 'promontorius' (Fig. 2.6).

A.



B.

C.

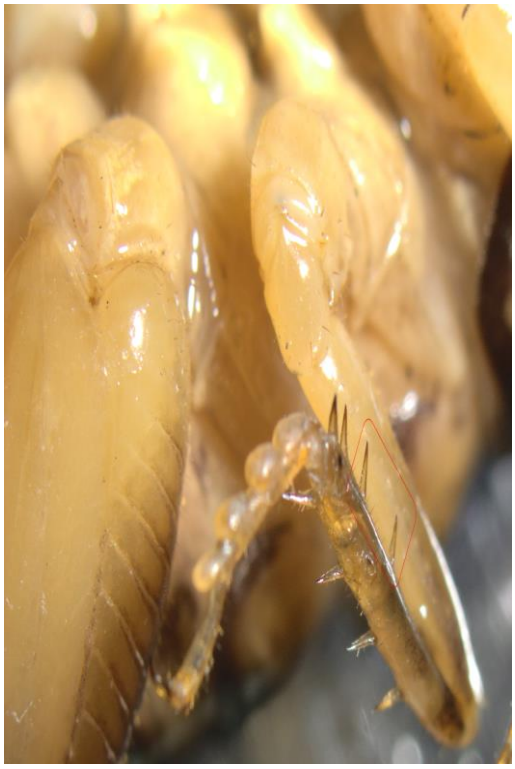


Fig. 2.5 Images showing some key morphological features of H. sp. 'promontorius'. **A.** Contrasting the basal glabrous segments of the antennomeres and hairy segments. **B.** The two spines close to the midpoint of the front tibia; **C.** The prolateral and retro-lateral spines of the mid-tibia; they are in pairs.

Figure 2.6 Female *Hemiandrus* sp. 'promontorius' and its eggs

2.4.2 Density and distribution of H. sp. 'promontorius'

2.4.2.1 Density of wētā in different habitats

Fig. 2.7 shows the density of wētā in each of the 15 sites sampled. The mean wētā density in January (summer) was not significantly different from that in November (spring). However, habitat significantly affected the density of the insect ($P < 0.001$). The highest mean density over the two seasons (i.e., summer and spring) was recorded in vineyards (3.3 individuals/m^2) whiles the lowest was in paddocks (0.02 individuals /m^2). Mean density in the latter habitat was not significantly different from that of shrublands (0.03 individuals/m^2) (Fig. 2.8).

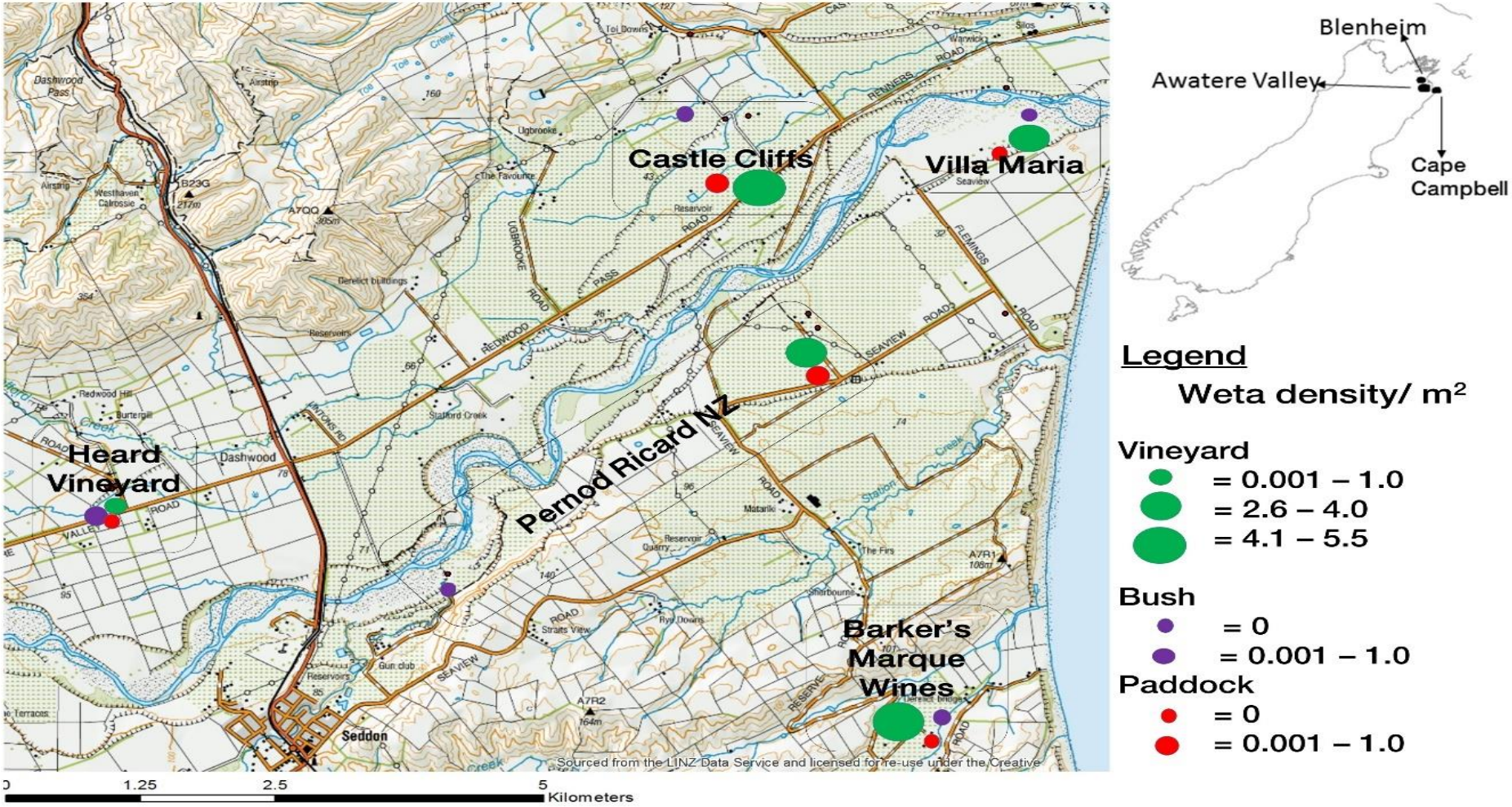


Figure 2.7 Map showing sites sampled in the Awatere Valley and wētā densities in the habitats

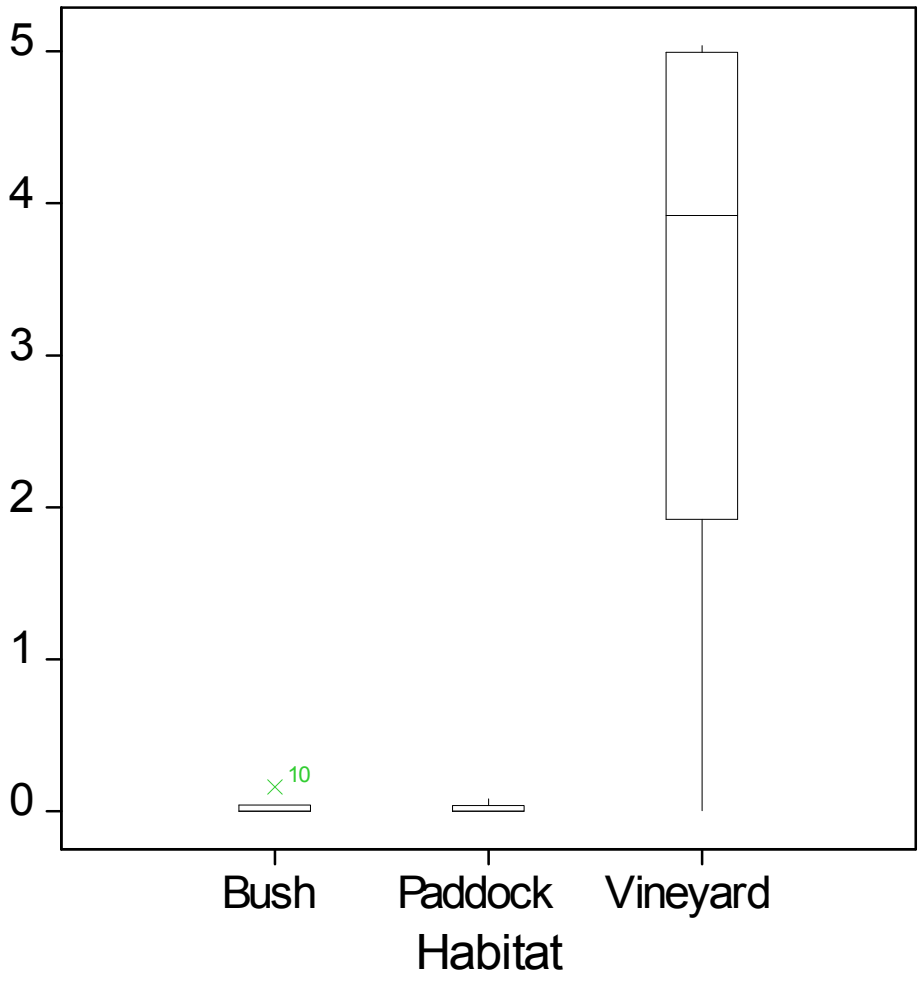


Figure 2.8 Boxplot showing density of wētā in different habitats in the Awatere Valley, Marlborough

2.4.2.2 Distribution and seasonal pattern in vineyards

Table 2.3 shows the wētā density at different dates and sampling positions within vineyards. Density was not significantly different between the edge and centre of the vineyards on any date. In May 2014, there was no significant difference between under-vine and inter-row densities. However, the density was significantly higher under-vines than in the inter-row from July, 2014 to January, 2015. The weighted mean density was significantly higher in January 2015 than in May, July and October, 2014.

Table 2.3 Mean density of *H.* sp. 'promontorius' at different locations in 6 vineyard blocks.

Period	Number of wētā/m^2		95% CI for mean difference	Number of wētā/m^2		95% CI for mean difference	Weighted mean density/m^2
	Edge (E)	Centre (C)		Under-vines (U)	Inter-rows (IR)		
May 2014	1.83	2.67	-0.84 ± 3.41 Ns	6.96	1.78	5.18 ± 5.72 Ns	3.51 b
July 2014	3.01	3.29	-0.28 ± 3.11 Ns	10.78	2.60	8.18 ± 4.75 *	5.32 b
October 2014	3.63	3.01	0.62 ± 2.09 Ns	18.74	0.00	18.74 ± 5.40 *	6.25 b
January 2015	8.95	6.77	2.18 ± 5.11 Ns	20.83	5.17	15.67 ± 10.70 *	10.39 a
Mean	4.58	4.05	0.53 ± 1.46 Ns	14.33	2.39	11.94 ± 5.61 *	6.37

Ns = not significant at 5% probability level; *= 5% significant; means in the last column with no letter in common are significantly different at the 5% level.

2.4.2.3 Egg laying and sex ratios

Of 74 adult wētā sexed in October 2014, 47.3% were males and the rest were females. In January 2015, 36.4% of the 77 insects were males and 36.7% (11) of those sexed in March 2015 were males. The observed proportion of males was not significantly different to that of females (P = 0.050; 95% CI for percentage of males = 33.76%–50.02%). The sex ratio in October was not significantly different from that in January (($\chi 2 = 1.4$) and March (($\chi 2 = 0.59$). There was no significance difference in sex ratio between January and March (($\chi 2 = 0.04$).

Forty-four of the 149 females collected from May to October, 2014 were found brooding eggs but none was found in January, 2015 (Fig. 2.9). The proportion of brooding females varied significantly with the seasons and was highest in July 2014 (40.3%) and lowest in January 2015 (zero) ($\chi 2 = 10.2$; $P < 0.01$). The proportion of females with eggs in October (25.4%) was not significantly different from that in July (40.3%) ($\chi 2 = 1.23$) and May (34.3%) ($\chi 2 = 0.21$) but these were significantly higher than in January 2015. The mean number of eggs per female was 55 ± 5 (n = 68).

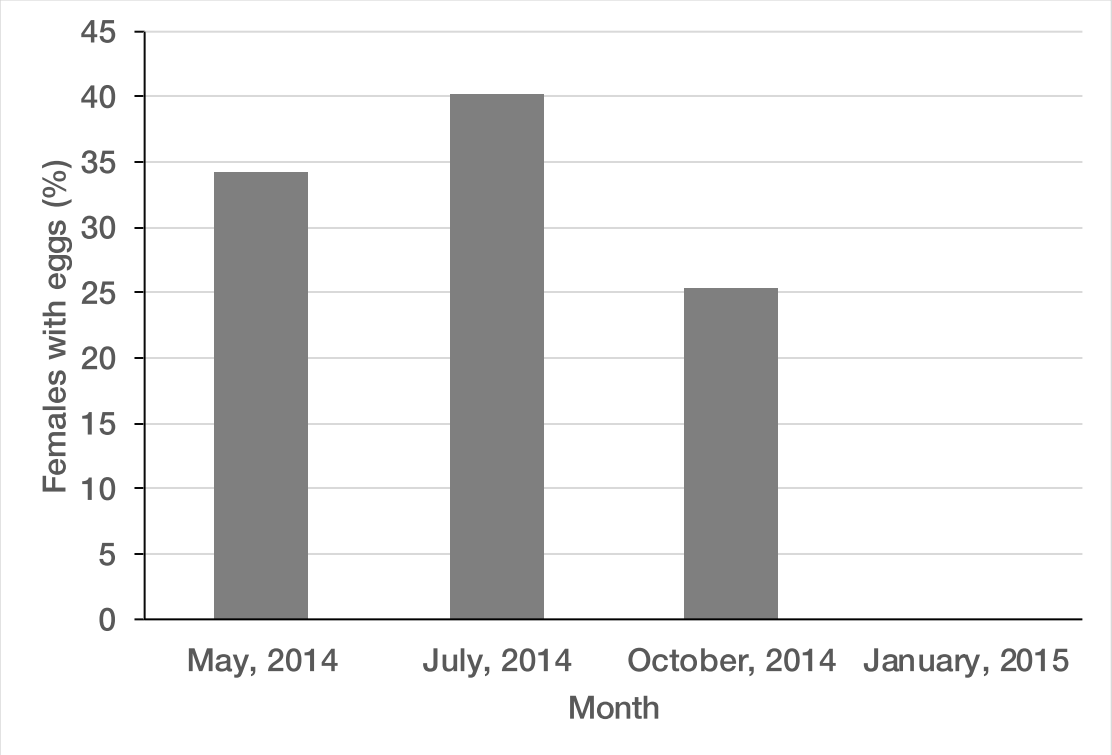


Figure 2.9 Proportion of female wētā brooding eggs in different months

2.4.3 Relationship between wētā density and soil properties

There was a significant positive relationship between wētā density and soil moisture (P = 0.018) (Fig. 2.10A). The latter accounted for 44% of the fitted regression model. Wētā density was significantly

and inversely related to soil compaction (P = 0.010; R^2 = 0.4972; Fig. 2.10B). Soil moisture content was significantly and inversely related to compaction (P<0.001; R^2 = 0.8116).

Wētā density was not significantly related to soil organic matter (P=0.127; R^2 =0.2173) or pH (P =0.540; R^2 =0.0387) (Fig. 2.10C–D). Similarly, there was no significant relationship between soil moisture and either organic matter content (P =0.250; R^2 =0.1299) or pH (P =0.211; R^2 =0.0098).

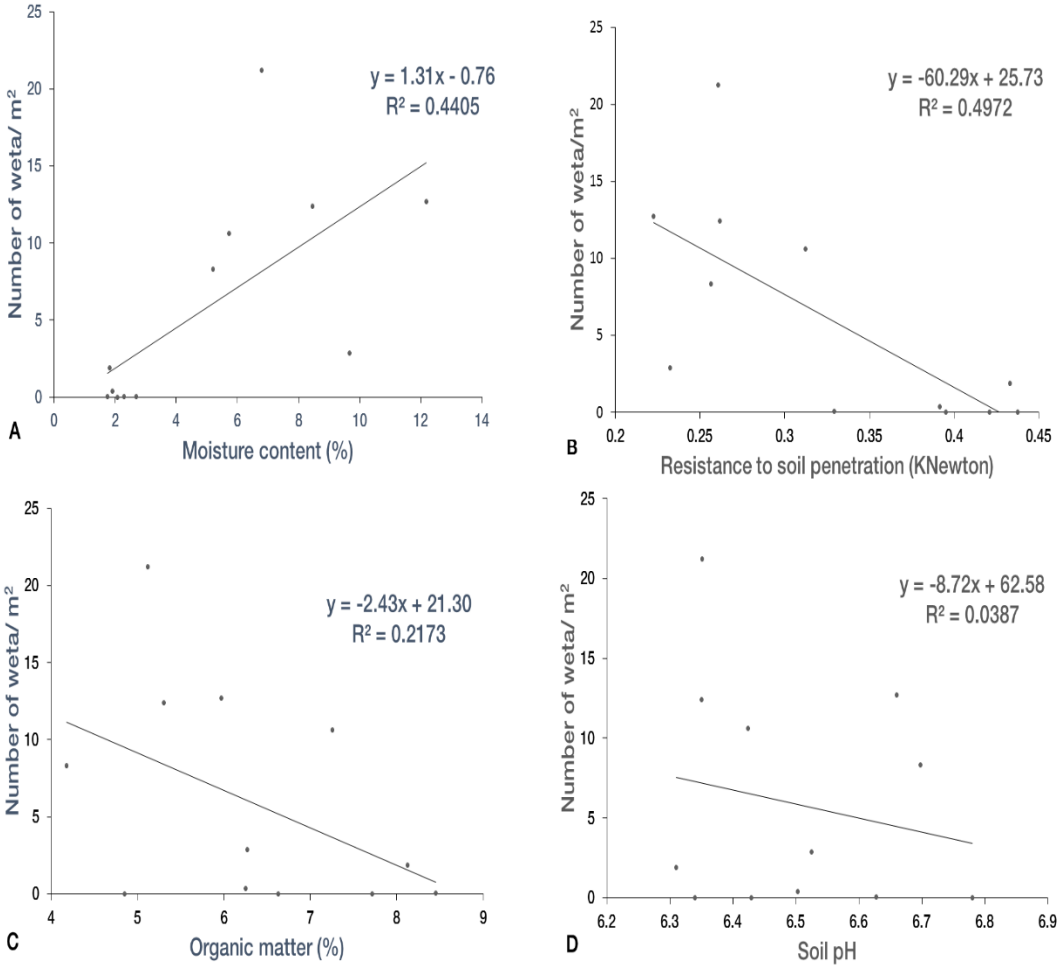


Figure 2.10 Relationship between the density of wētā and soil properties. **A,** Wētā density versus moisture content (%). **B,** Wētā density versus resistance to soil penetration (KNewton). **C,** Wētā density versus organic matter (%). **D,** Wētā density versus pH.

2.5 Discussion

2.5.1 Genetic and morphological identification of wētā

The mitochondrial gene, cytochrome oxidase *c* subunit 1 (CO1), is widely used as a standard barcode in identification and phylogenetic analysis of species in the animal kingdom (Hebert et al., 2003). Since its advent as a barcode region over a decade ago, thousands of species have been identified or phylogenetically analysed using this gene region (Kumar, Rajavel, Natarajan, & Jambulingam, 2007; Ojha et al., 2014; Witt, Threloff, & Hebert, 2006; Zhao, Gentekaki, Yi, & Lin, 2013). For identification of invertebrates, a 2% intraspecific divergence in this region is generally accepted as a threshold for delimiting species (Ball & Armstrong, 2006; Hebert et al., 2003). The accuracy of identifications is enhanced when a taxon has low divergences among individuals of the same species and high divergences among different species (i.e., high barcode gaps = ratio of inter- to intra- specific divergence (Zhao et al., 2013). In this work, intraspecific divergences lower than 2% existed between specimens collected from vineyards and also between the specimens used here and a sequence in Genbank submitted as *Hemiandrus* sp. 'promontorius' by Chappell et al. (2012). This confirmed that a single species of wētā was associated with vine damage in the Awatere Valley, Marlborough. However, DNA barcoding alone was thought to be insufficient in determining the identity of this species because the sequences generated from this work could only be compared the single *H.* sp. 'promontorius' sequence in Genbank. There were two other sequences in Genbank submitted as *H.* sp. 'promontorius' by Pratt et al. (2008), but these were later confirmed to be inaccurate (Steven Trewick, Massey University, New Zealand, pers. com.), thus they were not used for the phylogenetic analysis in this study.

In a recent work by (Taylor-Smith et al., 2016), the ground wētā, *H. maculifrons*, was found to previously have encompassed three different species - *H. maculifrons*, and two others. They therefore re-described *H. maculifrons* and named the other two as *H. luna* and *H. brucei*. Their work succeeds earlier taxonomic revisions by (Johns, 1997) which combined the two genera, *Zealandosandrus* and *Hemiandrus*, into the latter. With more of such taxonomic work, morphological keys can be easily used by non-taxonomists to establish the precise identity of ground wētā. In the meantime, there is a need for wētā taxonomists and scientist interested in barcoding to work on building accurate publicly available genetic databases for these insects and also to establish thresholds for delimiting wētā species. This will eliminate most errors in the identification of these insects. Until then, morphological keys and DNA barcoding should be used together when there is a need to identify this group of wētā.

The *H.* sp. 'promontorius' identified here, was first found between Marfells Beach (-41.7255°E, 174.2045°N) and Cape Campbell (-41.7372°S, 174.2760°E), Marlborough; both locations are close to the Awatere Valley (Johns, 2001). It was initially assigned an 'indeterminate' conservation status because of the paucity of information on its biology and distribution (Sherley, 1998). This has since changed to a 'not threatened' status following the availability of new knowledge about large stable populations of this insect (Trewick et al., 2016). These populations are however, still restricted to particular locations in the Marlborough region (Townsend et al., 2008; Trewick et al., 2012).

2.5.2 Density and distribution of wētā

Of three major habitats sampled here (paddocks, shrublands and vineyards), the density of this wētā was about 100 times higher in vine than in non-vine habitats. Because this species is omnivorous (Johns, 2001)(Johns 2001), habitat choice might have been influenced by availability of plant and animal components of its diet. The inter-rows of vineyards were sown and maintained with grasses. Weeds also grew between these grasses and occasionally under the vines where irrigation water sustains their physiological growth and functioning (Cifre, Bota, Escalona, Medrano, & Flexas, 2005; Dalley, Bernards, & Kells, 2006; Jones, 2004) even in the dry summer and autumn. Wētā and other arthropod herbivores therefore have access to plant food throughout the year. The former also preyed on some of the latter (e.g. Collembolla, Coleoptera, Diptera) to further satisfy its animal protein requirement (Cary, 1983; Van Wyngaarden, 1995; Wahid, 1978). The year-round availability of food probably contributed to the high wētā numbers in these vineyards. The arid nature of the non-vine habitats, especially in summer and autumn, potentially reduced the availability of food required for their survival. This may have contributed to the low wētā numbers recorded in those habitats.

The mean density of *H.* sp. 'promontorius' in vineyards was estimated at 3.0 and 6.4 /m^2 in the two studies reported herein. The absence of prior information on the density of this insect in the Awatere Valley or elsewhere makes deductions on any population change difficult. But the conversion of these lands from paddocks and/or shrublands (Gillinghan, 2012) to vineyards does not seem to have adversely affected their survival. This is because the estimated population size for this wētā was higher than the 1.8 and 3.0 /m^2, reported for the ground wētā, *H. maia* Taylor-Smith, 2013 and *H. electra,* Taylor-Smith, 2013, respectively in non-agricultural lands (Smith et al., 2013). Given the size of the population and the significant damage on vines, this species can be considered as a pest in the Awatere Valley vineyards.

A population of the same species has recently been observed causing damage in vineyards in the Wairau Valley, Marlborough 63 km north west of the Awatere Valley (Joanne Brady, Constellation Brands, pers. comm. 2015; P. M Johns, Canterbury Museum, pers. comm. 2015). It is most likely that this wētā was present in these two valleys before the vines were planted but their fe eding damage to the vines was not noticed initially because their numbers were low. The vines in the Awatere Valley were planted in the late 1980s but economic damage by this insect was first observed in the early 2000s. This probably suggests that their numbers have increased over time, resulting in their feeding damage becoming noticeable. Another species in this genus, *H.* 'horomaka' was reported as a pest in apricot orchards at Horotane Valley, Christchurch (Wahid, 1978). These observations could indicate that the feeding activity of wētā in the genus *Hemiandrus*, makes them potential pests when their native habitat is converted to agricultural land.

In the vineyards studied, *H.* sp. 'promontorius' was present throughout the year. This indicates that wētā is adapted to the routine seasonal vineyard management practices (Siqueira, Silva, & Paz-Ferreiro, 2014; Wardle, Nicholson, Bonner, & Yeates, 1999). Its density was, however, lower in autumn, winter and spring than in summer when most of the individuals recorded were nymphs. Similar density fluctuation has been observed in other univoltine insects in the order Orthoptera (Mariottini, De Wysiecki, & Lange, 2011) and this is an important determinant of the potential threat to agricultural crops by pest species.

In vineyards, wētā density was higher under-vines than in the inter-rows. The former was bare and there was sparse plant debris on the soil surface, unlike the inter-rows that were densely covered with a mixture of grass species (*Lolium perenne* Linn., *Festuca pratensis* Huds., *Poa annua* Linn.). Ground wētā preferentially make their burrows in open ground under shrubs, grasses and trees (Johns, 2001; Smith et al., 2013). The presence of large areas of bare soil under-vines therefore contributed to the high density of this species. Its density was, however, the same between the edges and centres of vineyards.

This wētā whose habitat has been converted to vineyards should be protected from viticultural practices (e.g., pesticide applications) that can potentially harm it and thus, change its threat status. The viticulture industry and Department of Conservation can work together to achieve this outcome.

2.5.2.1 Oviposition and sex ratios

In summer, male *Hemiandrus* exit their burrows and sit on leaves where they use pheromones to attract mates from long distances. At short ranges, males attract females by drumming their abdomen onto a substrate (Gwynne, 2004). After mating, female *H.* sp. 'promontorius' begin

ovipositing in March and this could extend into May depending on when mating occurred (J. Nboyine, pers. obs.). Eggs were seen in vineyards from May to early October without any obvious changes in density. Other ground wētā (e.g. *H.* sp. 'horomaka', *H. pallitarsis* etc.) have been found brooding eggs at similar periods (Gwynne, 2004; Wahid, 1978). The mean number of eggs per female in vineyards was 55. This was higher than the 30 eggs per female reported by Gwynne (2004) for the same species, possibly because observations in the current work were made on field populations rather than captive ones, as used by Gwynne (2004). In the present work, nymphs were first seen in late September for this species and their emergence was estimated to begin at least 5 months after oviposition, though eggs of other ground wētā can hatch after 4 months (Gwynne, 2004; Wahid, 1978).

The sex ratio did not differ from autumn, spring and summer. Previous studies of the sex ratio of wētā in the family Anostostomatidae Saussure 1859 mostly concluded that populations were either male- or female-biased. For example, the tusked wētā, *Motuwētā riparia* Gibbs, 2002*,* (McCartney, Armstrong, Gwynne, Kelly, & Barker, 2006) and the stone wētā, *Hemideina maori* (Pictet & Saussure, 1891) were female-biased (Joyce, Jamieson, & Barker, 2004) while populations of a ground wētā, *Hemiandrus maculifrons,* were male- biased (Chappell, Webb, & Tonkin, 2014). These ratios, however, were probably skewed due to sampling error (Wehi et al., 2011). This can be avoided when decisions on method and time of sampling are based on an analysis of species behaviour. For instance, sexually active male and female ground wētā actively exit their burrows for mating during the breeding season and will therefore be easily trapped or sighted during night searches (Chappell et al., 2014; Gwynne, 2004). Sex ratios estimated with these methods and at such periods are likely to be 50:50. After mating, males continue to exit their burrows and forage actively but the activity of females depends on their degree of maternal care. Those that exhibit maternal care (i.e. species with short ovipositor, e.g., *H.* sp. 'promontorius') are mostly occupied tending their eggs and seldom exit their burrow unlike species that do not show maternal care (i.e. species with long ovipositor) (Gwynne, 2004). Thus, females with a short ovipositor will be less frequently trapped or sighted than the males of the same species and its sex ratios will be erroneously skewed in favour of males when estimated during this period.

2.5.3 Relationship between wētā densities and soil properties

The density of wētā was higher at locations with low soil penetration resistance. Soils with high resistance to penetration are difficult to dig and are prone to flooding after precipitation due to reduced infiltration rate (Hamza & Anderson, 2005). Wētā will therefore require more force to burrow such areas. Such soils are also less well aerated and this does not support the survival of soil

organisms (Lipiec & Stepniewski, 1995). The wētā may have avoided these conditions. Generally, soils in the inter-rows required higher force of penetration than those under-vines. This was probably caused by farm machinery and by grazing farm animals being used for weed management (Hamza & Anderson, 2005; Lipiec & Stepniewski, 1995; Whalley, Dumitru, & Dexter, 1995).

Soil moisture was another important factor that determined the distribution of the wētā species studied here. Work on another ground wētā species, *H.* sp. 'horomaka', in apricots also found that it inhabited mainly moist areas (Wahid, 1978). This could be due to the influence of moisture on the availability of prey and the presence of its desired plant food (Brust & House, 1990; Chikoski, Ferguson, & Meyer, 2006; Mariottini et al., 2011; Powell, Berg, Johnson, & Warland, 2007). In addition, moisture is needed for egg development and hatching in ground wētā (Wahid, 1978) and in some grasshopper species that lay their eggs in the soil (Mariottini et al., 2011). Consequently, newly hatched nymphs do not migrate over long distances but build their burrows at close proximity resulting in increased density over time.

The density of this insect was not related to either soil organic matter or pH. These parameters were relatively uniform both within and between vineyards. The uniformity of pH values was because of the application of lime to soils (Baath et al., 1980) in vineyards prior to their establishment. In a related study, pH had no effect on the density of a soil burrowing cricket (*Gryllotalpa major* Saussure, 1874) (Hill, Deere, Fancher, Howard, & Tapp, 2009). Soil organic matter is also generally uniform for a given crop cover and cultivation practices on a farm (Burke et al., 1989; Parton, Schimel, Cole, & Ojima, 1987). These did not change within and between vineyards, and were therefore not correlated with wētā distribution.

Conclusions

In conclusion, this study provides fundamental information on the density and biology of *H.* sp. 'promontorius' in vine and non-vine habitats in the Awatere Valley. The conversion of the habitat of this insect into vineyards has not adversely affected its numbers. Densities were, in fact, higher in vine than non-vine habitats causing significant economic damage as they fed on vine buds. These findings should assist the Department of Conservation and vineyard managers to make informed decisions about the management of this species. Native species becoming agricultural pests after conversion of their natural habitat has been observed in many other taxa (Lefort et al., 2014). However, because wētā are iconic animals and this particular species is rare, it is essential to devise management measures that preserve the population while limiting damage to vines. For example, interventions could target only under-vine areas and/or those soils with low compaction and/or soil

moisture. Further work is needed to estimate the population size of this wētā in the Wairau Valley, Marlborough and other locations.

Chapter 3

Plant components of wētā diet in Awatere Valley vineyards

3.1 Abstract

Intensification of agriculture has led to monocultures over large areas of land, elevating many insects to the status of economic pests. Non-crop habitats, are sometimes deployed as trap crops to reduce pest damage. However, this requires knowledge of the most appropriate plant species to use. Here, ingested plant DNA in the faeces of an orthopteran pest, a wētā (*Hemiandrus* sp. 'promontorius'), 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. Polymerase chain reaction targeting the *rbc*L gene region were performed, followed by sequencing on the illumina MiSeq platform. The identities of plants in the diet of this insect were determined by comparing the sequences generated with those available in GenBank. A total of 30 plant families and 44 genera were detected. Only 57% of the taxa could be identified to the species level, while 100% could be identified at genus level. Species from the genera, *Vitis* sp., *Poa* spp., *Festuca* spp., *Anthoxanthum* spp., *Menyanthes* spp., *Garrya* spp. and *Tilia* spp. were the major ones (present in at least 50% of the faecal samples). The composition of the above plant taxa in faecal materials did not change significantly with sites or dates, which indicates high level of diet mixing throughout the year. Diet mixing is a common feeding behaviour among generalist insect herbivores and omnivores, as it ensures a balanced nutrient intake. Mitigating wētā damage to vine is therefore likely to benefit from enhancing vineyard plant diversity to include species that are favoured by wētā and which offset probable nutrient imbalance due to the dominance of grasses in vineyards.

Key words: **DNA, diet analyses, faeces, pest management, vineyards, New Zealand**

3.2 Introduction

Agricultural intensification has led to monocultures of high yielding plant species/cultivars over vast areas of land (Metcalf, 1994; Sandhu et al., 2016). This provides abundant resources for insects which feed on those monocultural species, elevating them to the status of economic pests (Altieri, 1999; Bianchi, Booij, & Tscharntke, 2006; Dent, 2000; 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 (Carvalho, 2006; Fernandez-Cornejo, Jans, & Smith,

1998; Schreinemachers & Tipraqsa, 2012). These practices have led to major biodiversity losses, unwanted adverse effects on the environment and to agriculture being called 'the largest ecological experiment on earth' (Rockström, Steffen, Noone, Persson, Chapin, et al., 2009; Rockström, Steffen, Noone, Persson, Chapin III, et al., 2009). Although the risks to human health and the environment from these chemicals have resulted in some evidence of shifts to more sustainable non-pesticide pest management practices (Brown, 1999; Ekström & Ekbom, 2011; Lewis, Van Lenteren, Phatak, & Tumlinson, 1997), most food production worldwide still relies heavily on high-input practices.

Alternative strategies, although still under-deployed, have the enhancement of functional farmland plant diversity as a key component (Gurr, Wratten, Landis, & You, 2016; Gurr, Wratten, & Luna, 2003; 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; Knapp & Řezáč, 2015; Landis, Wratten, & Gurr, 2000; Verkerk, Leather, & Wright, 1998). Non-crop vegetation in or around farmland may also attract, divert or intercept the targeted insect pest(s) and reduce their damage 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 (Holden, Ellner, Lee, Nyrop, & Sanderson, 2012; Moreau & Isman, 2012; Shelton & Badenes-Perez, 2006; Zhou, Chen, & Xu, 2010).

These pest management principles have been used worldwide in a variety of cropping systems including viticulture (Baša Česnik et al., 2008; Villanueva-Rey, Vázquez-Rowe, Moreira, & Feijoo, 2014). For instance, although vineyards are almost monocultures, it is common for at least one grass species to cover the inter-row areas (Lieskovský & Kenderessy, 2014; Ruiz-Colmenero, Bienes, Eldridge, & Marques, 2013). However, recent evidence has shown that grasses habour no more natural enemies of pest than does bare soil (Shields, Tompkins, Saville, Meurk, & Wratten, 2016). 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 (Altieri, Ponti, & Nicholls, 2005; Berndt et al., 2002; Berndt, Wratten, & Scarratt, 2006; Shields et al., 2016).

In addition, inter-row vegetation and any surviving weeds could act as alternative food sources for generalist insect pests thereby potentially reducing economic damage. This however, is not always the case in practice. As in other cropping systems, the presence of non-crop vegetation does not necessarily result in reduced pest damage to the main crop (Berndt et al., 2002; Paredes, Cayuela,

Gurr, & Campos, 2015; Shelton & Badenes-Perez, 2006; Villa, Santos, Mexia, Bento, & Pereira, 2016). This is because the success of this approach to pest management hinges on identifying and deploying the 'right' non-crop species (Gurr et al., 2016; Landis et al., 2000; Simon, Bouvier, Debras, & Sauphanor, 2010).

Generally, identification of candidate trap-plant species may involve the time-consuming method of 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 methods of gut content or faecal analysis are available (e.g., microhistological analysis, near infra-red reflectance spectroscopy, stable isotopes etc.), but they often lack taxonomic resolution. On the other hand, recent advances in DNA barcoding, combined with high-throughput DNA sequencing, make it possible to identify and describe the composition of an animal's diet with high precision (Pegard et al., 2009; Pompanon et al., 2012; Soininen et al., 2009; Valentini, Pompanon, & Taberlet, 2009). Hence, the current work aimed at analysing ingested plant DNA in the faeces of a generalist orthopteran pest, a ground wētā (*Hemiandrus* sp. 'promontorius': Anostostomatidae), in New Zealand vineyards to help identify appropriate candidate plant species for inclusion in its management strategy, e.g., as potential trap plants.

Although many insect pests emerge when they are introduced to a new habitat, the novel association that results from the introduction of new crop plants can also lead to native species becoming pests (Lefort, Worner, Rostas, Vereijssen, & Boyer, 2015). This is the case for the wētā *H.* sp. 'promontorius' which is native to New Zealand but has become a pest in vineyards (See chapter 2). This wētā is present in vineyards throughout the year but significant damage to vines occurs only at budburst (Joanne Brady, Constellation Brands, pers. comm., 2015)). Information on other plants on which it feeds is essential for developing and deploying non-pesticide management practices for this pest. The aim of this study was also to contribute to existing knowledge on why generalist feeders can be pests, even when the crop itself may not dominate the agricultural area e.g., vines with grasses in the inter-rows. To describe the diet of *H.* sp. 'promontorius' in New Zealand vineyards, individual wētā were collected from six vineyard blocks over four seasons and their faeces screened for plant DNA using high throughput DNA sequencing.

3.3 Materials and methods

3.3.1 Wētā collection sites

Six vineyard blocks in three different vineyards were sampled in the Awatere Valley, Marlborough, New Zealand (see Table 2.1). These 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 herbicides to kill all weeds. Maintaining bare under-vine areas in spring is key to minimizing frost damage to vine buds. Pine tree (*Pinus* spp. L.) hedges bounded at least one side of each sampled block (Creasy & Creasy, 2009; Joanne Brady, Constellation Brands, pers. com.).

3.3.2 Sampling wētā from vineyards for faecal analysis

Wētā were randomly sampled from each of the six vineyard blocks over four seasons. The sampling periods were July 2014, October 2014, January 2015 and April 2015. 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 (Fig. 3.1). The arenas were stored at room temperature (20 °C) for 24 h, after which the insects were released. 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 dishes (Fig. 3.2) and stored at -80 °C pending DNA extraction.

Figure 3.1 Plastic arena lined with double layer tissue paper for collecting wētā faeces

Figure 3.2 Petri dishes containing wētā faeces

3.3.3 DNA extraction

DNA was extracted from 72 out of a total of 160 faecal samples (i.e., three randomly selected pellets per site per season) using a Zymo Research Fecal DNA MicroPrep™ kit. This was because the tagging solutions recommended by the manufacturer is based on 96-wells and can therefore only accommodate 96 samples. However, few wells are required for quality control (positive and negative controls). It was therefore not possible to analyse more than three samples per block and per season on one plate as processing a second plate would double the cost of the analysis. The manufacturer's protocol was followed with slight modifications. To extract DNA from wētā faeces, 500 µl lysis solution was pipetted into 72 individual BashingBead™ lysis tubes each containing faeces. All the faecal samples of an individual wētā were put into each tube because they weighed 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 minutes, followed by centrifuging at 10,000 g for 1 minute. The supernatants (400 µl) were transferred to Zymo-Spin™ IV spin filters in collection tubes and centrifuged at 7,000 g for 1 minute. Faecal DNA binding buffer (1,200 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 minute. This was followed by pipetting 200 µl DNA pre-wash buffer and 500 µl faecal DNA wash buffer to the columns and centrifuging for 1 minute 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 seconds 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 minutes before centrifuging at 8,000 g for 1 minute. The purified DNA was then amplified through polymerase chain reaction (PCR).

3.3.4 PCR and electrophoresis

The universal primer pair (*rbc*L19 and *rbc*LZ1; Poinar et al., 1998) which amplifies a short fragment of the ribulose bisphosphate carboxylase large subunit (*rbc*L) chloroplast DNA gene region was used to perform PCR aimed at detecting ingested plant DNA in wētā faeces. Primers were designed to include the recommended overhang adapters for illumina sequencing (see Table 2). 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 $MgCl_2$ (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 min, and a final elongation at 72 °C for 10 min. A positive (mixture of plant DNA) and negative (PCR grade water) control were included in each of the PCRs 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 2ng/µL. Unique molecular identifiers (MID) were added to each sample before high-throughput DNA sequencing on an illumina MiSeq platform using the 200 × 200 paired end protocol as recommended by the manufacturer. These last two steps were performed by New Zealand Genomics Ltd, Auckland, New Zealand.

Table 3.1 General plant primers targeting plastid *rbc*L DNA

Primer	Direction	Plastid DNA region	Sequence (5' – 3')
*rbc*L19	Forward	*rbc*L gene	AGATTCCGCAGCCACTGCAGCCCCTGCTTC
*rbc*LZ1	Reverse	*rbc*L gene	ATGTCACCACAAACAGAGACTAAAGCAAGT

3.3.5 Data analysis

Sequences generated by Miseq sequencing were collapsed into unique Molecular Operational Taxonomic Units (MOTUs) with a one base mismatch allowance. Merged sequences from the Miseq run that were shorter than 150bp were discarded and any sequence with more than one expected error in the sequence was also excluded. To make the downstream analysis faster, non-unique sequences were removed. Singleton Operational Taxonomic Units (OTUs) were then discarded, and the unique sequences were clustered using a 97% identity threshold. Chimeric sequences were then removed using a *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 BLAST. Identifications accepted as correct matches and used for subsequent analyses in this study were those with query coverage > 80%, identity > 97%, and E- values < 1.0×10^{-29}. The accepted identifications were further cross checked with a database of plants present in New Zealand (Allan Herbarium, 2000). Sequences with no match (according to the above criteria) 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.

Because read counts (the number of sequences) from digested food items may not be an accurate representation of the amount of food ingested (Valentini *et al.*, 2009b), the data were converted into presence (1)/ absence (0) before performing statistical analyses. A conservative approach in which 'presence' was assigned to MOTUs that occurred at least four times in each faecal material, while 'absence' was assigned to those that were detected in less than four times was and only present in one faecal sample (Valentini, Pompanon, et al., 2009).

Genera which were detected in at least 50% of the samples analysed were considered as major food items and were subjected to further statistical analyses. These major taxa were *Vitis* sp. (vines); *Poa* spp., *Festuca* spp., *Anthoxanthum* spp. (grasses); *Epilobium* spp., *Menyanthes* spp. (weeds); *Garrya* spp. and *Tilia* spp. (trees). They were categorized 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. These categorizations were considered necessary for determining the effect of agricultural practices on the insect's feeding behaviour.

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 3 faecal

samples for each sampling unit) and logit link function were chosen for these analyses. The response variables were the taxa, 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) at the 5% probability level.

Significant differences between the proportions of groups, subgroups and genera of plants were determined by computing the 95% confidence intervals (CI) of their mean difference.

3.4 Results

3.4.1 High-throughput DNA sequencing

A total of 8,096,949 paired end reads were successfully merged, and 7,413,745 reads remained after the removal of low quality reads. The size range of the amplicons was 88 – 153 bp, excluding adapters primers. Of these reads, 7,408,085 were subsequently clustered into 1,950 OTUs at a 97% threshold. These OTUs were later searched against the BLAST nucleotide database which identified 1,495 MOTUs.

The total number of OTUs with query coverage > 80%, identity > 97%, and E- values < 1.0×10^{-29} was 182. This reduced to 125 OTUs after checking for records of the genera that they matched in New Zealand plant database. The identified taxa belonged to 30 plant families and 44 genera. Of the families detected, Poaceae and Caryophyllaceae comprised seven and five different genera, respectively. The families Rosaceae, Solanaceae, Lamiaceae and Asteraceae each comprised two genera. The remaining 24 families displayed only one genus each (Table 3.2).

The total number of sequences for the genera, *Vitis, Poa, Festuca, Epilobium, Tilia, Cordia* and *Urtica,* was 3,461,197 and they accounted for approximately 97% of all the sequences generated in this work (Table 3.2). That corresponded to an average of 49,020 sequences per faecal sample for those genera.

Only *c*. 57% of the 44 plant genera could be identified to the species level.

Table 3.2 Plant taxa identified from wētā faeces and the proportion of each genus in faecal material

Family	Genus	Species	Detection rate	Description
Vitaceae	*Vitis*	*V. vinifera* L. (1753)	1.0	Vines
Poaceae	*Poa*	*P. pratensis* L. (1753)	1.0	Grass
	Festuca	*F. arundinacea* Schreb. (1771)	1.0	Grass
	Anthoxanthum L. (1753)		0.59	
	Elymus L. (1753)		0.23	
	Eleusine	*E. indica* (L.) Gaertn. (1788)	0.04	
	Dactylis L. (1753)		0.07	
	Sacciolepis	*S. indica* (L.) Chase (1908)	0.01	
Onagraceae	*Epilobium*	*E. montanum* L. (1753)	0.50	Willow-herb
Malvaceae	*Tilia* L. (1753)		0.94	Tree
Caryophyllaceae	*Silene* L. (1753)		0.03	Weed
	Amaranthus	*A. tricolor* L. (1753)	0.17	
	Atriplex	*A. patula* L. (1753)	0.10	
	Suaeda Forssk. (1775)		0.11	
	Chenopodium	*C. murale* (L.) S. Fuentes, Uotila & Borsch (2012)	0.03	Goosefoot
Urticaceae	*Urtica*	*U. dioica* L. (1753)	0.34	Perennial nettle
Rosaceae	*Potentilla* L. (1753)		0.28	Strawberries
	Prunus		0.21	Shrub
Convolvulaceae	*Ipomoea*	*I. batatas* (L.) Lam.	0.01	Sweet potato
Musaceae	*Musa*	*M. acuminata* Colla (1820)	0.10	Banana/plantain
Amaryllidacea	*Allium*	*A. tuberosum* Rottler ex Spreng. (1825)	0.06	Onions
Asteraceae	*Senecio* L. (1753)		0.19	Groundsels/ragworts

	Crepis L. (1753)		0.01	Hawksbeard
Menyanthaceae	*Menyanthes*	*M. trifoliata* L.	0.70	Buckbean
Brassicaceae	*Camelina*	*C. sativa* (L.) Crantz	0.46	False flax
Cucurbitaceae	*Cucumis*	*C. melo* L. (1753)	0.11	Gourd plant
Primulcaceae	*Anagallis*	*A. arvensis* L. (1753)	0.40	Scarlet pimpernel
Fagaceae	*Quercus* L. (1753)		0.34	Tree
Nothofagaceae	*Nothofagus*	*N. nitida* (Phil.) Krasser (1896)	0.01	Tree
Garryaceae	*Garrya* Douglas ex Lindl.		0.81	Tassel bush/ shrub
Geraniaceae	*Erodium*	*E. trifolium* (Cav.) Guitt. (1963)	0.06	Weed
Lamiaceae	*Nepeta*	*N. faassenii* Bergmans ex Stearn (1950)	0.14	Catmint
	Prunella	*P. vulgaris* L.	0.03	Selfheal
Boraginaceae	*Cordia* L.		0.43	Shrub/tree
Orobanchaceae	*Pedicularis* L.		0.34	Broomrape
Lauraceae	*Machilus* Nees.		0.13	Tree
Alstroemericiaceae	*Luzuriaga*	*L. parviflora* (Hook.f.) Kunth (1850)	0.29	Herb
Salicaceae	*Populous*	*P. nigra* L.	0.24	Tree
Podocarpaceae	*Dacrydium* Sol. Ex G.Forst. (1786)		0.14	Shrub/tree
Prumnopityaceae	*Prumnopitys*	*P. taxifolia* (Sol. ex D. Don) de Laub. (1978)	0.01	Tree
Polygonaceae	*Fagopyrum* Mill. (1754)		0.16	Buckwheat
Ranunculaceae	*Ranunculus* L. (1753)		0.03	Buttercups/spear worts
Solanaceae	*Iochroma* Benth. (1845)		0.06	Tree/shrub
	Solanum	*S. lycopersicum* L. (1753)	0.17	

3.4.2 Plant materials detected in wētā faeces

The proportions of faecal material that tested positive for the genera *Vitis* sp.*, Poa* spp.*, Festuca* spp., *Anthoxanthum* spp., *Menyanthes* spp., *Garrya* spp. and *Tilia* spp. did not change significantly with date and site. The only significant difference was found with *Epilobium* spp., the occurrence of which changed with date only ($P = 0.028$). Detection of this spring-flowering annual weed was highest in April and lowest in July. There were no significant differences between the detection rate of this species between July, October and January (Fig. 3.3).

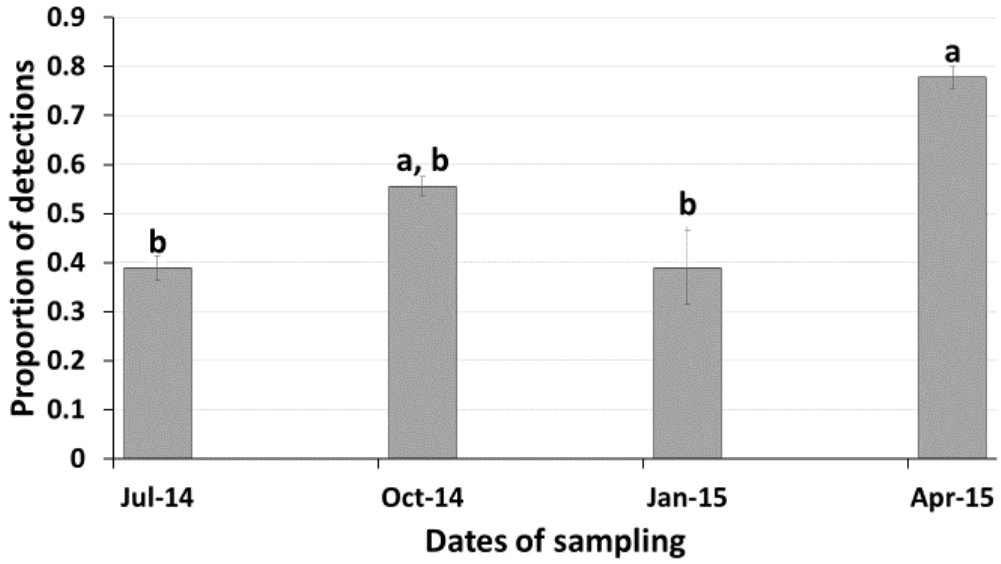


Figure 3.3 Proportion of wētā frass testing positive for *Epilobium montanum* detected at different dates of sampling. Bars with no letters in common significantly different at the 5% level of significance.

There were significant differences between the proportional detections of the genera occurring in ≥ 50% of the materials analysed, irrespective of site and date ($P < 0.001$). The detections of each of the genera *Vitis* sp.*, Poa* spp. and *Festuca* spp., were proportionately higher than that for *Anthoxanthum* spp. (95% CI: 0.32 – 0.53, $P < 0.05$), *Epilobium* spp. (95% CI: 0.19 – 0.45, $P < 0.05$), *Menyanthes* spp. (95% CI: 0.19 – 0.45, $P < 0.05$) and *Garrya* spp. (95% CI: 0.11 – 0.28, $P < 0.05$). There were no significant differences between the detections of *Vitis* sp.*, Poa* spp.*, Festuca* spp. or *Tilia* spp. in the faecal materials. Similarly, there were no significant differences between the proportions of *Anthoxanthum* spp., *Epilobium* spp. or *Menyanthes* spp. detected (Fig 3.4).

Pairwise comparisons of the mean proportional detections of the different categories of plants showed that, vines (1.00) occurred more often than trees (*Tilia* spp, *Garrya* spp.) (0.44) (95% CI: 0.07 – 0.18, $P < 0.05$) and weeds (0.30) (95% CI: 0.33 – 0.48, $P < 0.05$). The mean proportion of grasses (*Poa* sp., *Festuca* spp., *Anthoxanthum* spp.) (0.86) were significantly higher than weeds (*Epilobium* spp., *Menyanthes* spp.) (95% CI: 0.21 – 0.31, $P < 0.05$) (Fig 3.4). Trees were similarly significantly higher than weeds (95% CI: 0.20 – 0.37, $P < 0.05$).

Also, the mean detection rate of cultivated (grasses, vines) plants (0.46) were significantly higher than uncultivated (weeds + trees) plants (0.37) (95% CI: 0.16 – 0.23, $P < 0.05$).

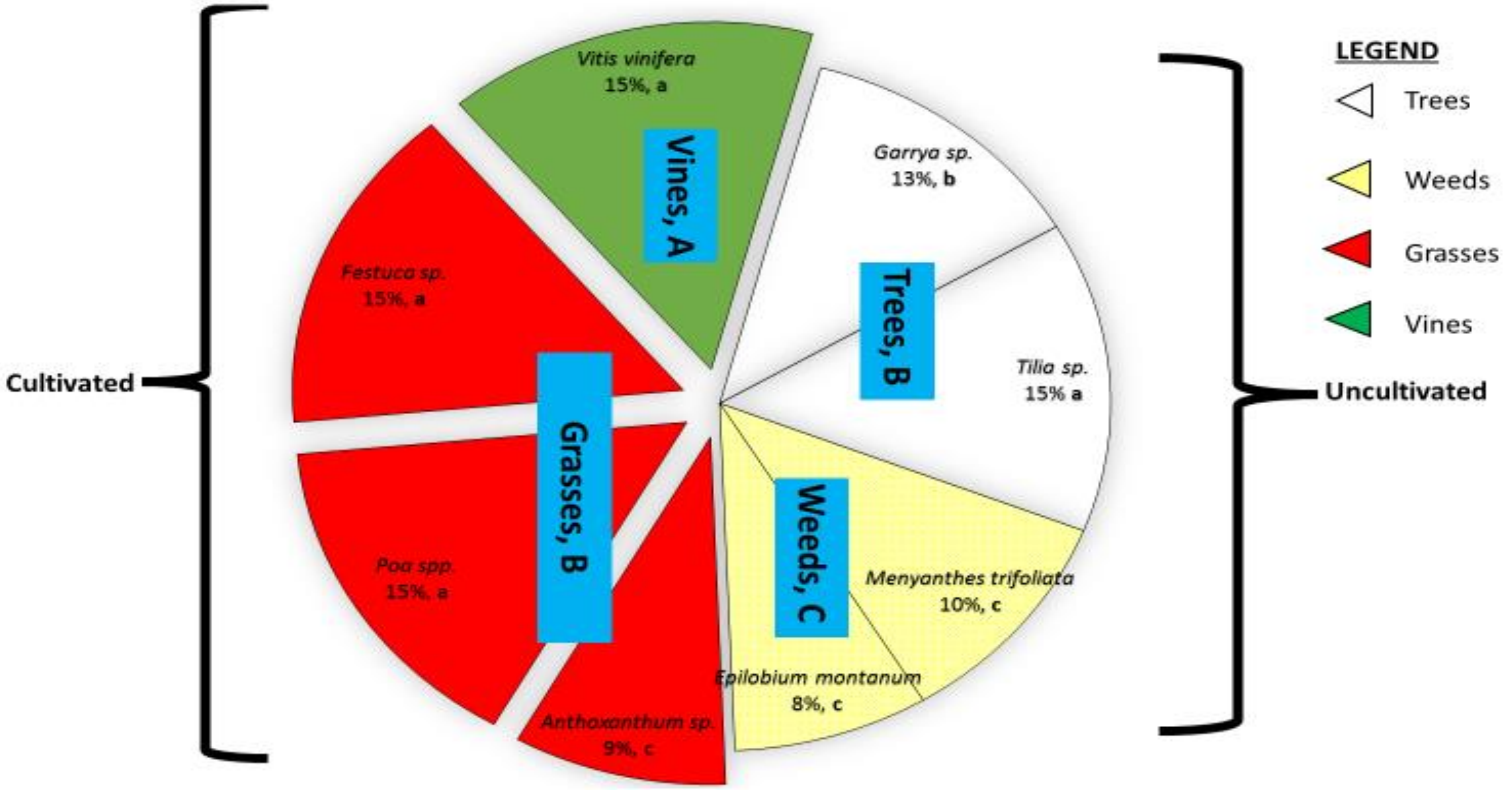


Figure 3.4 Proportion of the major plant genera detected through molecular analysis of wētā frass. Means with different case letters are significantly different at 5% probability threshold. Trees = *Garrya* sp. + *Tilia* sp.; weeds = *Epilobium montanum* + *Menyanthes trifoliata*; grasses = *Poa* spp. + *Festuca* sp. + *Anthoxanthum* sp.; vines = *Vitis vinifera*; cultivated = grasses + vines; uncultivated = trees + weeds.

3.5 Discussion

3.5.1 High-througput DNA sequencing

DNA barcoding of plants can be challenging. This is because of the absence of a single standard barcode region that is sufficiently variable within it to discriminate among species and yet conserved across all groups of land plants (Group et al., 2009; Hollingsworth, Graham, & Little, 2011; Newmaster, Fazekas, & Ragupathy, 2006; Newmaster, Fazekas, Steeves, & Janovec, 2008). Hence, combining the 2-locus (*rbc*L + *mat*K) is generally recommended because of their recoverability, sequence quality and level of species discrimination (Group et al., 2009; Hollingsworth et al., 2009). The *rbcL* region is easily recovered while the *matK* region has a high discriminatory power. However, the latter can be difficult to PCR amplify using existing primer sets (Hollingsworth et al., 2011). For analyses of the diet of herbivores, the choice of a barcode region requires knowledge of the range of potentially consumed species (i.e., taxonomic coverage) and the taxonomic resolution of the barcode region (Pompanon et al., 2012; Taberlet et al., 2012). The P6 Loop of the *trn*L intron and/or the *rbc*L region are usually recommended, but not *mat*K, because the former regions are easily amplified and are well conserved for land plants, thus allowing for a high taxonomic resolution (Pompanon et al., 2012; Valentini, Miquel, et al., 2009; Valentini, Pompanon, et al., 2009). In addition, these regions are relatively short (12 – 134 bp and 115 bp respectively), which makes them more likely to be amplified from degraded DNA samples such as faeces and gut content (Pompanon et al., 2012).

Of the two recommended regions, the *rbc*L gene was targeted although the *trn*L intron is the most frequently reported in herbivore diet studies (Soininen et al., 2009; Staudacher, Wallinger, Schallhart, & Traugott, 2011; Wallinger et al., 2013). This was because an earlier study comparing the *rbc*L gene and the P6 Loop of the *trn*L intron on the Illumina Miseq system showed higher sequencing success for the *rbc*L region (Burgess et al., 2011; Kajtoch, 2014). Major advantages of the *trn*L intron over the *rbc*L gene, which has resulted in its wide use, are the availability of large databases and its high taxonomic resolution to the species level (Pompanon et al., 2012; Taberlet et al., 2012). However, this study was conducted in an agricultural system where sequences of the range of plants present are largely available in public databases. In general, there are about 225,323 *rbc*L and 238,989 *trn*L nucleotide sequences recorded in the Genbank. Of these, 1018 *rbc*L and 303 *trn*L sequences were submitted by workers in New Zealand (www.ncbi.nml.nih.gov/ Accessed on 1 March, 2017). Thus, the probability of detecting fauna, especially those unique to New Zealand, is

higher when *rbc*L gene region is targeted. Also, the sequencing system and the agricultural habitat used improved the detection success.

Approximately 57% of the MOTUs generated from wētā frass could be identified at the species level using *rbc*L. This was higher than the 50% reported by Valentini et al. (2009) using the *trn*L intron to analyse the diet of various herbivores – mammals, birds, mollusc and insects. Similar studies using ABI (Applied Bioscience Inc.) Sanger sequencing or 454 sequencing system (Rosche) showed higher numbers of plants being identified to the species level with *trn*L intron than with the *rbc*L gene (Staudacher et al., 2011; Taberlet et al., 2007; Valentini, Miquel, et al., 2009). However, in a comparison between illumina MiSeq and Sanger sequencing, Kajtoch (2014) recommended the use of primers targeting the *trn*L intron over *rbc*L if the Sanger sequencer was to be used. Hence, the sequencing system should be considered along with the choice of a barcode region.

For scientists seeking to develop pest management strategies based on an understanding of a generalist insect's diet in an agricultural system, this study recommends the use of primers targeting the *rbc*L gene region. In non-agricultural systems where the potential range of plants present in an insect's diet is probably much more diverse and potentially unknown, the two gene regions (*rbc*L and *trn*L intron) combined in a multi-locus approach is often recommended (Staudacher et al., 2011). However, where the range of plants expected is present in databases for both gene regions, the *rbc*L gene region seems to produce better results on the illumina MiSeq platform because of its high sequencing success. This notwithstanding, most identifications are accurate to the family level only. Beyond this, the accuracy level reduces.

3.5.2 Implications for pest management

Wētā in the genus *Hemiandrus* are usually omnivores, feeding on a diverse range of plant and animal materials (Cary, 1983; Johns, 2001; Wahid, 1978). Diets comprising a mixture of plant and/or animal species is a common feeding behaviour among generalist orthopterans and other omnivore arthropods (Coll & Guershon, 2002; Raubenheimer & Jones, 2006). This gives such insects a better nutrient balance than is possible by feeding on a single plant taxon, resulting in increased growth and survival (Bernays, Bright, Gonzalez, & Angel, 1994; Berner, Blanckenhorn, & Körner, 2005; Coll & Guershon, 2002; HaÈgele & Rowell-Rahier, 1999). Also, 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 & Agrawal, 2012; Bernays et al., 1994). The current work only focused on the plant-based diet of *H.* sp. 'promontorius'. A total of 30 different families and 44 genera of plants were identified from faecal samples.

Dicotyledonous weeds were rare in the vineyards studied here. However, they were detected in the diet of every wētā collected in spite of the unlimited availability of grasses and vines. Tree species were similarly detected in all faecal materials analysed. Studies of the diet of generalist insect feeders indicate that, when they are restricted to an unbalanced diet, they compose their food intake to limit the extent to which nutrients are occurring in excess or in deficit (Behmer, 2009; HaÈgele & Rowell-Rahier, 1999; Raubenheimer & Simpson, 2003). 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 (Hannaway et al., 1999; Lledó, Rodrigo, Poblaciones, & Santamaria, 2015). Proteins are a major requirement of the diet of *Hemiandrus* spp. (Johns, 2001; Smith, 2015b; Van Wyngaarden, 1995). Being an omnivore, this insect can balance its protein intake by preying on other insects of vines. These were however, killed by the regular applications of insecticides. Therefore, sustainable intake of protein for this wētā may rely on balanced feeding on weeds and tree species when grasses and vines are mature.

Weed species are sometimes deliberately used to provide shelter, nectar, alternative host and pollen needed to attract and enhance the 'fitness' of natural enemies for insect pests' population regulation in many habitat diversification pest management strategies on farms (Altieri, 1999; Bianchi et al., 2006; Brown, 1999; Holden et al., 2012; Norris & Kogan, 2000; Simon et al., 2010). The findings here suggest that, this approach to pest management could have the added advantage of reducing damage to the main crop (e.g., vines) by generalist insect herbivores and omnivores such as wētā which may use weeds as alternative foods (Araj, Wratten, Lister, & Buckley, 2009).

Conclusions

In conclusion, the current work examined how the results of faecal DNA analyses could potentially contribute to developing non-pesticidal pest management strategies, thus reducing the high pesticide input in most modern agriculture. Primers targeting a short fragment of the *rbc*L gene region were used to successfully identify the range of plants eaten by wētā, at least to the genus level. Approximately 55% of the plants could be identified to the species level. This was higher than that reported in previous studies (Taberlet et al., 2007; Valentini, Miquel, et al., 2009) and was because they used pyrosequencing instead of illumine Miseq platform. A wide variety of plant families were found in the diet of this insect, in spite of grasses being abundant in vineyards. This feeding behaviour is common among generalist insect 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 should use 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 economic crops. If these plants are potential weeds, they can be removed, for example with herbicides, once the pest damage period has passed. However, many non-crop plants in vines or other crops deliver a wide range of ecosystem services including regulating the population of pest species (Araj et al., 2009; Sandhu et al., 2016; Shields et al., 2016). Managing non-crop plants in agriculture could be key to achieve 'sustainable intensification'.

Chapter 4 Management of wētā in vineyards

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4.1 Abstract

Soil-dwelling insect pests are important in crop production. They are often unnoticed until damage occurs because of their subterranean behaviour; this makes their control difficult, even with pesticides. Here, the efficacy of different combinations of under-vine and inter-row treatments for managing a soil-dwelling orthopteran pest, wētā (*Hemiandrus* sp.), in vineyards was investigated in two seasons. This insect damages vine buds, thus reducing subsequent grape yield. The under-vine treatments comprised pea straw mulch, mussel shells, tick beans, plastic sleeves on vine trunks (the existing standard control method) and control (no intervention), while inter-rows contained either the existing vegetation or tick beans. Treatments were arranged in a randomized complete block design with 10 replicates. Data were collected on wētā densities, damage to beans and components of vine yield. The under-vine treatments significantly affected all variables except the number of shoots per bud. In contrast, none of the variables was significantly affected by the inter-row treatments or their interaction with under-vine treatments, apart from wētā density. At the end of the experiment, wētā density in the shell treatment was *c.* 58% lower than in the control. As a result, there was *c.* 39% significant yield increase in that treatment compared to the control. Although the under-vine beans and sleeves treatments increased yield, there were no reductions in wētā density. With under-vine beans, the insect fed on the bean plants instead of vine buds. Thus, yield in that treatment was *c.* 28% higher than in the control. These results demonstrate that simple agro-ecological management approaches can reduce above-ground damage by soil-dwelling insects.

Key words: cover crops, grapevine yield, soil-dwelling insects, pest management, vineyards, yield loss

4.2 Introduction

Pests that spend the major part of their development living in the soil can be economically important in crop production (Blossey & Hunt-Joshi, 2003; Brown & Gange, 1989; Jackson & Klein, 2006; Klein, 1988). Their feeding activity can cause extensive damage to plants (Blossey & Hunt-Joshi, 2003; Wood & Cowei, 1988). For instance, larvae of the beetles *Melolontha* sp. Fabricius,

1775, *Holotrichaia* sp. Hope, 1837, *Leucopholis* sp. Dejean, 1833, *Oryctes* sp. Illiger, 1789, etc. are subterranean and feed on plant roots, while their adults are polyphagous, feeding on leaves and sometimes, unripe fruits (Hill, 1983; Jackson & Klein, 2006; Keller & Zimmermann, 2005). Other taxa such as mole crickets (*Gryllotalpa* sp. Latreille, 1802), crickets (*Acheta* sp. Linnaeus, 1758, *Brachytrupes* sp. Serville, 1839) and larvae from some lepidopteran families (e.g., Hepialidae, Noctuidae, Pyralidae, Castiniidae) live in burrows in the soil and exit these at night and damage plants by feeding on young shoots (Hill, 1983; Wylie & Martin, 2012).

The management of these pests is difficult because they are subterranean and their presence is not usually detected until the plants are damaged (Jackson, 1999; Musick, 1985). Many farmers, therefore, rely on prophylactic chemical use to prevent damage but this can result in problems of pesticide residues in plants, outbreaks of secondary pests and insecticide resistance (Jackson, Alves, & Pereira, 2000; Lacey & Shapiro-Ilan, 2008). Research aimed at developing alternative approaches for managing soil-dwelling insect pests has focused on the use of entomopathogenic microbes such as fungi (*Beauveria bassiana* (Balsamo) Vuillemin (1912), and *Metarhizium anisopliae* (Metchnikoff) Sorokin (1883)), nematodes (*Heterorhabditis* sp. Poinar, 1976, *Steinernema* sp.) and bacteria (*Bacillus* sp. Cohn, 1872, *Serratia* sp. Bizio, 1823) (Ansari, Brownbridge, Shah, & Butt, 2008; Jackson & Jaronski, 2009; Lacey & Shapiro-Ilan, 2008; Pereault, Whalon, & Alston, 2009; Shah & Pell, 2003). However, this strategy has some limitations, such as entomopathogenic and microbial products being unable to reach the target pest in the soil, as well as the failure of most of the applied microbes to survive in the soil environment (Jackson, 1999). Therefore, there is a need to explore other approaches for managing these pests.

In perennial crops (e.g., orchards and vineyards), mulch applied to the understorey soil enhanced the abundance of generalist predators and other potential biocontrol agents and these were considered to reduce the population of subterranean stages of some insect pests (Addison, Baauw, & Groenewald, 2013; Brown & Tworkoski, 2004; Campos-Herrera, El-Borai, & Duncan, 2015; Mathews, Bottrell, & Brown, 2002, 2004; Robertson, Kettle, & Simpson, 1994). Also, weed management strategies such as sowing centipedegrass (*Eremochloa ophiuroides* (Munro)) in the understoreys of peach orchards proved effective for controlling the soil-dwelling stages of *Conotrachelus nenuphar* (Herbst, 1797) (Coleoptera: Curculionidae), by serving as a physical barrier to emergence of its adults (Akotsen-Mensah, Boozer, & Fadamiro, 2012). Trap cropping has been used to effectively manage many insect pests including those living in the soil (e.g., *Agriotes* sp. Eschscholtz, 1829 (Coleoptera: Elateridae)) in perennial fruit crops (Bugg, Dutcher, & McNeill, 1991; Bugg & Waddington, 1994; Landl & Glauninger, 2011; Liang & Haung, 1994). It involves planting a

crop that is more attractive to the pest as either a food source or oviposition site than is the main crop (Shelton & Badenes-Perez, 2006; Zehnder, Gurr, Kuhne, et al., 2007). However, this strategy is knowledge-intensive and if the choice of trap plant is not carefully done, deploying it could increase the occurrence of other pests with or without reducing that of the target one (Bugg & Waddington, 1994; Shelton & Badenes-Perez, 2006).

Overall, these strategies have mostly been effective against the soil-dwelling stages of coleopteran and lepidopteran insect pests but evidence for their efficacy on burrowing insects in the order Orthoptera is not conclusive. This work therefore studied the efficacy of two types of mulch (pea straw (*Pisum sativum* L.) and mussel shells (*Perna canaliculus* Gmelin, 1791)) and a cover crop (*Vicia faba* Linn. var. *minor* (Fab.)) for the management of a soil-dwelling orthopteran insect pest, wētā (*Hemiandrus* sp. 'promontorius' (Johns, 2001)), in vineyards. This insect damages vines (*Vitis vinifera* L.) by feeding on either the compound bud or the primary bud inside the compound bud at budburst (Joanne Brady Constellation Brands NZ pers. comm, 2014). The latter leads to low yield from clusters growing on shoots arising from the inferior secondary buds, or sometimes no yield or canes for the next season if the whole compound bud is destroyed (Creasy & Creasy, 2009; Joanne Brady Constellation Brands NZ pers. comm, 2014). Damage is currently managed by tying plastic sleeves around vine trunks. These are slippery and make it difficult for wētā to access the tender growing buds on the canes. However, this management technique is labour intensive and costly and sleeves often need to be repaired/replaced, leading to further costs.

4.3 Materials and methods

4.3.1 Study period and site

This study was conducted in the Awatere Valley, Marlborough, New Zealand in the 2014/15 and 2015/16 seasons. The vine cultivar studied was Sauvignon Blanc. The work took place at a different site in each season in vineyards belonging to Constellation Brands, New Zealand. The experiments were established in September and the grapes harvested in March in each season. These vineyards were subjected to conventional management practices, involving the use of pesticides for weeds, insect pest and disease management. For insect pests, methoxyfenozide (with trade name Prodigy) was applied at flowering for caterpillars of the leafroller complex (*Epiphyas postvittana* (Walker, 1863), *Ctenopsuestis* spp., *Planotortrix* spp.). This insecticide had no effect on wētā and its application occurred outside the period wētā damage in vineyards. Karate (lambda-cyhalothrin) is usually applied in the headlands of vineyards in response to the flight of grassgrubs (*Costelytra*

zealandica (White, 1846)), but this was not sprayed in the vineyard blocks used for this experiment because of its potential effect on the study insect.

The climate in the Awatere Valley is more extreme than in other parts of the Marlborough region. It has mean daily minimum and maximum temperatures of 7.5 and 18.1 ^{O}C, respectively. This valley has an annual rainfall range of 557 – 1042 mm (http://www.wineresearch.org.nz/category/weather-data/awatere-valley-dashwood-weather-data/, Accessed on 20 July, 2016).

4.3.2 Experimental layout

Treatments formed a 5 x 2 factorial structure, with two treatment factors, "under-vine" and "inter-row" (see Fig. 4.1). The under-vine treatment factor comprised 5 levels: control (no intervention), pea straw mulch, tick beans, mussel shells and plastic sleeves (Figure 4.2). The inter-row factor had 2 levels: the existing ryegrass (*Lolium perenne* L.)-dominant vegetation and tick beans. The 5 x 2 = 10 treatments were randomly allocated to 10 plots within each of 10 blocks, in a randomized complete block design (Table 4.1). 'Plot' refers to an under-vine area and the two inter-row areas on either side of it in a bay, while 'block' consisted of all the plots in a vine row. A bay comprised four vine plants which were bounded by two wooden posts. Vines had a within-row spacing of 1.8 m and a between-row spacing of 2.4 m. The under-vines and inter-rows in each bay occupied areas of 5.76m^2 (= 7.2 x 0.8) and 28.8m^2 (= 7.2 x (2 x 2.4 – 0.8)), respectively. The plots within the blocks were separated by a distance of 7.2 m (the length of a bay), while blocks were 4.8 m apart (2 buffer rows). In all, there was a total of 100 plots (i.e., 10 plots / block and 10 blocks). Figure 4.1 shows the experimental layout for the 2014/15 season. The treatments were re-randomized in 2015/16.

Table 4.1 List of under-vine and inter-row treatment pairs

Under-vine treatments	Inter-row treatments
Control (Bare ground/ no intervention)	Existing ryegrass-dominant vegetation
Mussel shells	Existing ryegrass-dominant vegetation
Pea straw mulch	Existing ryegrass-dominant vegetation
Tick beans (UVTB)	Existing ryegrass-dominant vegetation
Plastic sleeves on stem	Existing ryegrass-dominant vegetation
Bare ground	Tick beans (IRTB)
Mussel shells	Tick beans (IRTB)
Pea straw mulch	Tick beans (IRTB)
Tick beans (UVTB)	Tick beans (IRTB)
Plastic sleeves on stem	Tick beans (IRTB)

*Bare ground means glyphosate was used to remove all the weeds; UVTB = Under-vine tick beans; IRTB = Inter-row tick beans

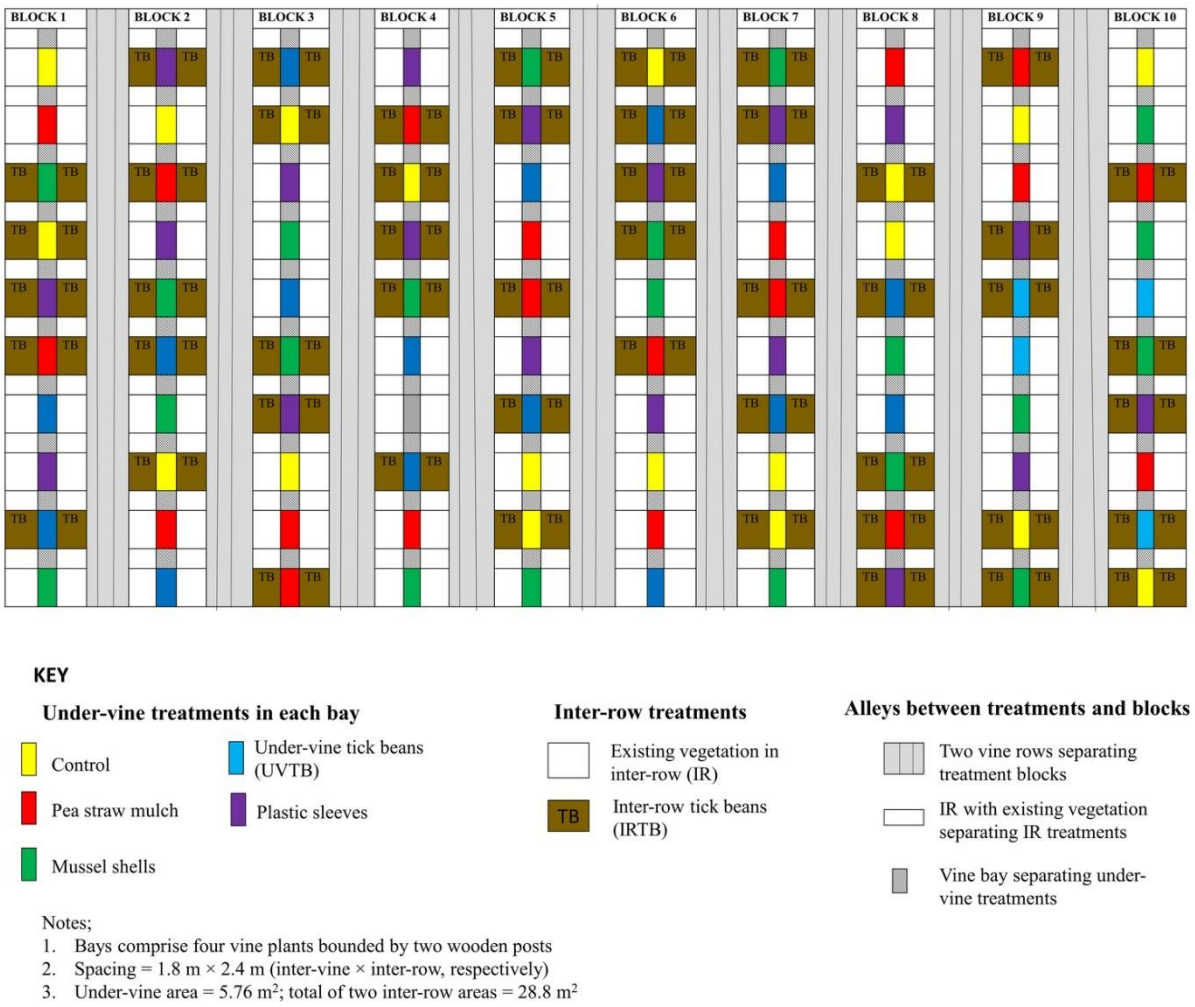


Figure 4.1 Experimental layout in the vineyard in the 2014/15 season, as 10 blocks of a 5 x 2 factorial. UVTB = Under-vine tick beans; IRTB = Inter-row tick beans

A. 

B. 

C.

D.

Figure 4.2 Some of the treatments tested for wētā management in vineyards. A. Inter-row tick beans; B. Under-vine mussel shells; C. Under-vine pea straw mulch; D. Plastic sleeve on vine trunk

Tick beans were used as a cover crop because results from preliminary laboratory bioassays showed a high preference for this species by the wētā (Smith, 2015a). The seeds were sown at a rate of 135kg per ha. Previous studies have shown that application of mulches in perennial crops increases

the diversity of their associated arthropod assemblage to include pests' natural enemies, and that this could be exploited in pest management (Addison et al., 2013; Brown & Tworkoski, 2004; Mathews et al., 2004). This was the rationale for the inclusion of pea straw as a mulch treatment here. Mussel shells were included because of their potential as a physical barrier to wētā exiting their burrows. The straw and shells were spread to completely cover the 5.76 m^2 under-vine area in each replicate to a height of 0.10 m.

The inter-row treatment, existing ryegrass-dominant vegetation, paired with either bare ground or plastic sleeves served as untreated or treated controls, respectively.

4.3.3 Maturity indices measurement

Wine quality depends on certain measurable properties of wine grapes referred to as maturity index. This index is in influenced by factors such as soil moisture, canopy temperature, yield etc. and these factors could in turn be affected by the treatments tested (Creasy & Creasy 2009; Keller 2010). Hence, in each season, approximately 150 grape berries were sampled from each treatment and replicate at harvest into zipped plastic bags (Fig 4.3A). They were crushed by hand and the juice from each treatment and replicate transferred into labelled 50 ml screw top plastic tubes (Fig 4.3B). The samples were immediately placed in refrigerator (i.e., below 5 ^{o} C) to stop any further chemical reaction that will affect subsequent measurements. In all, 100 samples (comprising 10 from each treatment) were analysed.

The maturity indices of the samples were determined by first centrifuging each sample, after which 4.8 ml of the clear solution from each sample was placed into a WineScan™ FT 120 model (Fig 4.3C). This machine analyses up to 120 samples at a time and measures brix, pH, titratable acid (TA), malic acid, free alpha-amino nitrogen (FAN), ammonia, glucose, fructose, ethanol, volatile acids, lactic acid, glycerol and total acid.

A. 

B. 

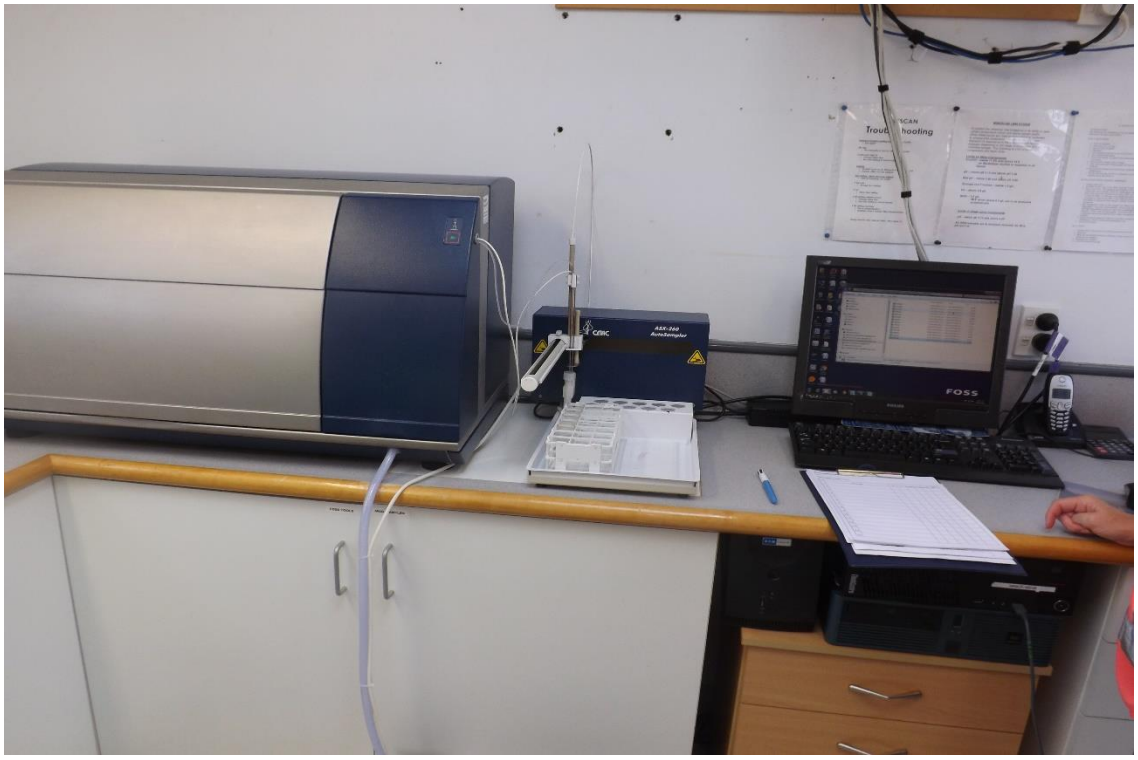
C. 

Figure 4.3. Measurement of maturity indices. A. Grape berries sampled into zipped plastic bag; B. Juice from crushed berries; C. WineScan WineScan™ FT 120.

4.3.4 Data collection

The two middle vines in each bay were assessed for number of buds laid down per vine, number of shoots/bud, clusters/shoot, number of grape bunches/vine, bunch weight (g) and grape yield (expressed in t/ha), while initial and final wētā densities were measured in the area between those two vines. Wētā feeding damage (%) was recorded on tick bean plants located in the under-vine and inter-row areas between the same two mid-vines in each plot.

Initial wētā density was estimated by sampling the under-vine areas of bays in the rows immediately opposite (i.e., to the right) the experimental plots. This was to avoid disturbing the wētā in the latter. Earlier studies of this pest found its density under vines to be relatively spatially uniform (See chapter 2). Hence, the density in the sampled bays was assumed to be similar to that in the experimental plots. To estimate this insect's density, the top 5 mm of soil between the two mid-vines in each sampled bay was scraped off to expose all burrows in that area. The burrows were counted, after which three of them per bay were excavated with a shovel to a depth of 300 mm. The soil was spread on the ground and carefully searched to count the insect. Data were expressed as the number of wētā-occupied burrows in an area of 1 m^2. Wētā density at the end of the experiment was estimated for all treatments and replicates as above. The shells, mulch and beans between the

two middle vines were carefully removed before scraping off the top soil as above for the final density estimates.

The number of buds on the canes of each vine were counted before budburst, while the number of shoots and clusters (inflorescences) were counted after budburst. The data collected were then used to compute the ratios of numbers of shoots per bud and clusters per shoot.

Tick bean damage was estimated for under-vine and inter-row areas by counting the number of bean plants with wētā feeding damage in a 1.44m^2 (= 1.8 m x 0.8 m) area. This was expressed as a percentage of the total number of plants within the area.

4.3.5 Data analysis

The data from each season were subjected to an analysis of variance (ANOVA) for a 5 (under-vine) x 2 (inter-row) factorial laid out in 10 randomised blocks. Means were separated using their least significant difference (LSD) at a 5% probability level. For data on wētā density, the effect of the treatments was determined by computing the logarithmic ratio of final to initial density before performing an ANOVA on it. This ratio measures the change in density due to the treatment effects.

To combine the results over the two seasons, a randomized complete block ANOVA was performed, using the 10 treatment means for each variable measured in each trial, as a 5 (under-vine) x 2 (inter-row) factorial with 2 blocks (= trials). Treatment means were again separated using their LSDs.

4.4 Results

In general, for all variables measured, the main effect of inter-row and the under-vine × inter-row interaction were not statistically significant, with two exceptions which are described later. Therefore, the results reported here focus on the main effect of the under-vine treatments.

4.4.1 Number of buds laid down and effects of under-vine management on components of yield

The mean numbers of buds laid down/vine at the start of the trial were 31.8 (± 1.32 S. E.) and 38.2 (± 1.48 S. E.) for the 2014/15 and 2015/16 seasons, respectively. There were no significant differences between treatments for the numbers of buds laid down in each season or for the results of their combined analysis.

Similarly, the number of shoots/bud was not significantly affected by the under-vine treatments in either seasons (P = 0.345 and 0.406 for 2014/15 and 2015/16, respectively) or in the combined

analysis results (P = 0.512). However, the overall mean number of shoots/bud in 2014/15 (0.77) was significantly lower than in 2015/16 (0.98) (P < 0.001).

There was, however, a significant main effect of under-vine treatments on the number of clusters/shoot in 2014/15 (P < 0.001) and 2015/16 (P < 0.001). Combining the means of the two seasons also showed a significant under-vine treatment effect (P < 0.001). The number of clusters/shoot in the shell treatment was approximately 1.3 times higher than that in the control (Fig. 4.4A). There were no significant differences between the number of clusters/shoot in shell, sleeves or under-vine tick bean (UVTB) treatments. The control and straw mulch treatments were not significantly different from each other in terms of the number of clusters/shoot. The overall mean of this variable in 2015/16 (1.60) was not significantly different from that in 2014/15 (1.70) (P = 0.083).

The mean bunch weight was significantly affected by the under-vine treatments in 2014/15 (P = 0.006). In contrast, there was no significant under-vine treatment effect for this variable in 2015/16 (P = 0.290). The combined analysis showed a significant main effect of under-vine treatments (P = 0.017). The mean bunch weights in UVTB, sleeves and shell treatments were *c.* 8 – 16% higher than in the control (P = 0.017) (Fig. 4.4B). The overall mean bunch weight in 2015/16 (105.00 g) was significantly higher than in 2014/15 (80.20 g) (P < 0.001).

There was a significant under-vine treatment effect for the number of bunches/vine and total grape yield in both seasons, and in the combined results. Yield was approximately 28, 30 and 39% higher in UVTB, sleeves and shell treatments, respectively, compared to the control (Fig. 4.4D). The number of bunches per vine also increased significantly by *c.* 22 – 37% in those treatments compared with the control (Figs. 4.4C & D). The overall mean grape yield and number of bunches/ vine were significantly higher in 2015/16 than in 2014/15 (P < 0.001).

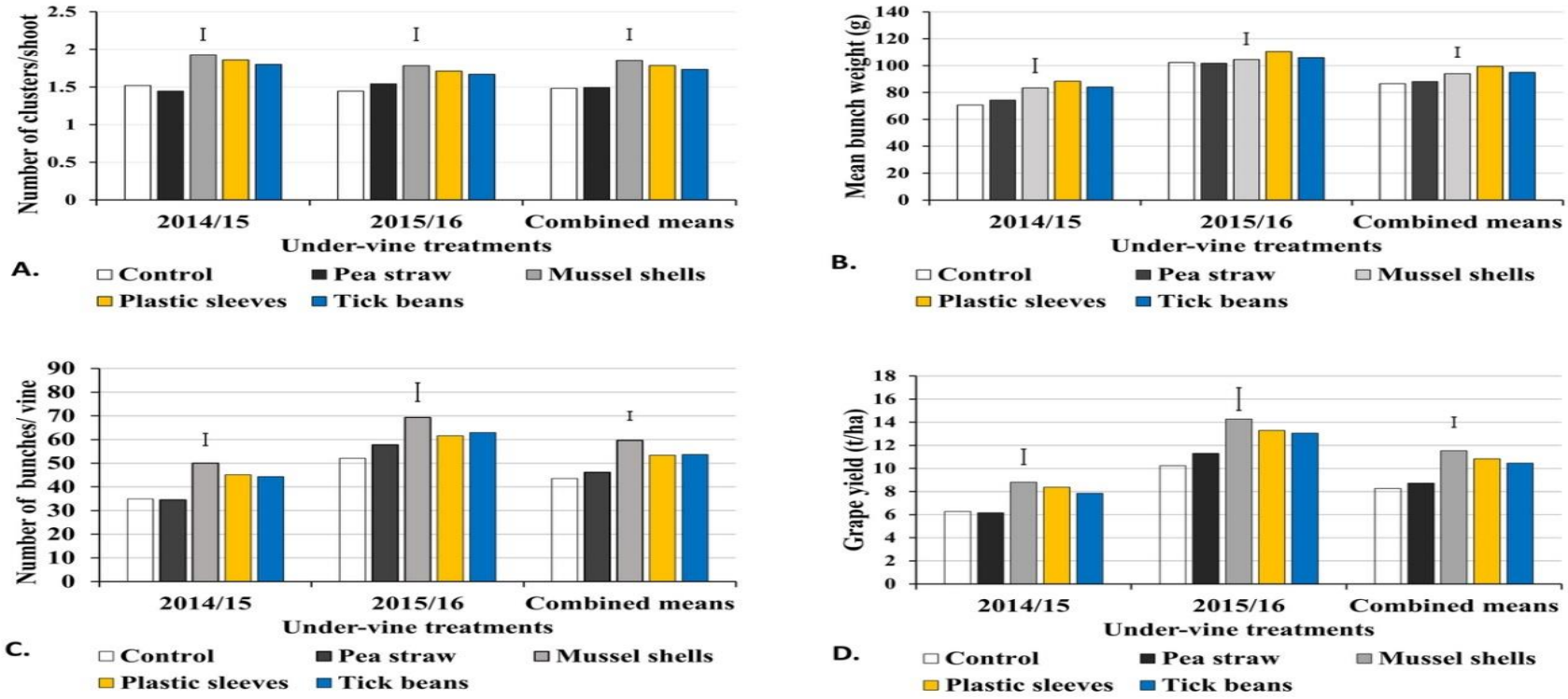


Figure 4.4 Main effect of under-vine wētā management strategies on components of yield in 2014/15 and 2015/16 seasons and their combined means. Bars represent LSD at 5% level of probability. **(A)** Number of clusters per shoot; **(B)** Mean bunch weight (g); **(C)** Number of bunches/vine; **(D)** Grape yield (t/ha)

4.4.2 Effects of under-vine management on wētā density

In both seasons, initial wētā densities were not significantly different between the treatments (Table 4.2). The density at the start of the experiment was approximately 1.10 and 1.60 wētā/m^2 for the 2014/15 and 2015/16 seasons, respectively. The final density was, however, affected by the inter-row treatments, but in 2014/15 ($P = 0.016$) only. The density was higher when the inter-rows were sown with beans than when the existing vegetation was maintained.

There was also a significant main effect of under-vine treatments for final wētā density in both seasons and in the results of the combined analysis. Among the under-vine treatments, final density was significantly lower in the shell treatment than in the control, straw mulch, UVTB and sleeves treatments (Table 4.2). However, there were no significant differences between the control and straw mulch, UVTB and sleeves treatments.

The change in wētā density (i.e., $\log_{10}$ final/initial wētā density) in each season and their combined results showed a significant under-vine treatments effect. This change was significantly higher in the shell treatment than in the others. There were no significant differences among the control, straw mulch, UVTB and sleeve treatments for their change in density. In 2015/16, there was a significant interaction effect for change in wētā density ($P = 0.043$). In that season, there was a significant 73% reduction in wētā density when shells were used under vines and beans were sown in the inter rows. In contrast, wētā density decreased by only 20% when shells were used under vines and the existing vegetation was maintained (Table 4.2).

The initial and final wētā densities were significantly lower in 2014/15 than in 2015/16. However, the extent of density changes was not significantly different between the seasons ($P = 0.992$; Table 4.2).

Table 4.2 Effect of management on density of wētā in vineyards

Under-vine treatments	Inter-row treatments	Mean wētā density/m^2 in 2014/15			Mean wētā density/m^2 in 2015/16			Combined mean of wētā density/m^2		
		Initial	Final	[1]Log_{10} (Final/initial)	Initial	Final	[1]Log_{10} (Final/initial)	Initial	Final	[1]Log_{10} (Final/initial)
Control	Existing vegetation	0.98	0.99	0.041 (1.10)	1.67	1.35	-0.042 (0.91)	1.32	1.25	-0.001 (1.00)
Pea straw	Existing vegetation	1.07	0.90	-0.071 (0.85)	1.72	1.06	-0.296 (0.51)	1.39	0.97	-0.160 (0.69)
Mussel shells	Existing vegetation	0.97	0.32	-0.535 (0.29)	1.49	0.96	-0.096 (0.80)	1.23	0.71	-0.316 (0.48)
Tick beans	Existing vegetation	1.29	1.13	-0.017 (0.96)	1.82	1.32	0.056 (1.14)	1.56	1.34	0.019 (1.04)
Plastic sleeves	Existing vegetation	1.10	0.92	-0.089 (0.81)	1.70	1.72	0.055 (1.14)	1.34	1.39	-0.017 (0.96)
Control	Tick beans	1.15	1.13	-0.046 (0.90)	1.46	1.65	0.012 (1.03)	1.31	1.43	-0.017 (0.96)
Pea straw	Tick beans	1.06	1.17	0.116 (1.31)	1.63	1.40	-0.069 (0.85)	1.35	1.22	0.024 (1.06)
Mussel shells	Tick beans	0.92	0.49	-0.292 (0.51)	1.49	0.50	-0.576 (0.27)	1.21	0.47	-0.434 (0.37)
Tick beans	Tick beans	1.27	1.28	0.041 (1.10)	1.48	1.47	-0.014 (0.97)	1.37	1.36	0.024 (1.06)
Plastic sleeves	Tick beans	1.17	1.08	-0.014 (0.97)	1.77	1.60	0.063 (1.16)	1.47	1.39	0.013 (1.03)
Mean		1.10	0.94	-0.087	1.62	1.30	-0.091	1.36	1.15	-0.09 (0.87)
[2]LSD (5%)		0.42	0.32	0.25	0.78	0.59	0.28	0.24	0.51	0.33
[3]LSE (5%)		0.30	0.23	0.18	0.55	0.42	0.80	0.17	0.36	0.23
P- values										
Main effects										
Under-vine (UV)		0.246	< 0.001	< 0.001	0.913	< 0.001	< 0.001	0.060	0.004	0.020
Inter-row (IR)		0.703	0.016	0.098	0.513	0.740	0.403	0.440	0.677	0.804
Interaction effect										
UV × IR		0.944	0.984	0.407	0.946	0.287	0.043	0.590	0.609	0.695
Significance of mean wētā density in 2014/15 versus 2015/16 season										
								< 0.001	0.002	0.992

Figures in brackets are back transformed means; [1]Log10 (final/Initial) = change in wētā density; [2]LSD (5%) = Least significant difference at 5% probability level; [3]LSE (5%) = Least significant effect at 5% probability level – if a log_{10} ratio of final to initial density is greater in magnitude than the LSE (5%), then the change in density is significantly different from zero.

4.4.3 Wētā feeding damage to tick beans

The extent of damage to tick beans was significantly different among the treatments in each season (Table 4.3). However, the combined analysis of the two seasons' means of wētā damage to beans showed a significant treatment effect only at the 10% probability threshold (P = 0.055). The "UVTB only" treatment was the most damaged while the "inter-row tick beans (IRTB) only" and "Pea straw +IRTB" treatments were the least affected. The extent of feeding damage among IRTB, UVTB + IRTB, shells + IRTB, mulch + IRTB and sleeves + IRTB treatments was not significantly different. The damage to beans in 2014/15 was significantly lower than that in 2015/16 (P = 0.008; Table 4.3).

Table 4.3 Wētā feeding damage (%) on tick beans in 2014/15 and 2015/16 seasons

Under-vine treatments	Inter-row treatments	Mean wētā feeding damage (%) on tick beans in different seasons		Combined mean feeding damage (%)
		2014/15	2015/16	
Tick beans only	Existing vegetation	79.6	85.0	82.3
Control	Tick beans	50.9	73.7	62.3
Tick beans	Tick beans	66.9	74.3	70.6
Mussel shells	Tick beans	61.3	71.5	66.4
Pea straw	Tick beans	51.4	70.6	61.0
Plastic sleeves	Tick beans	63.5	72.3	67.9
Means		62.3	74.6	68.42
LSD (P = 5%)		16. 5	7.5	12.8
P- value		0.014	0.004	0.055
Significance of mean feeding damage in 2014 versus 2015 season				
P- value				0.008

4.4.4 Maturity indices

The maturity indices brix, pH, TA, malic acid, FAN, ammonia, glucose and fructose were not significantly affected by the treatments tested in each of the seasons (P > 0.05). Similarly, combined analyses of the two seasons' data did not show significant treatment effect. However, there were significant differences between 2014/15 and 2015/16 for the measurements of all indices, except TA (Table 4.4).

Table 4.4 Effect of season on grape maturity

Season/ maturity indices	Brix (°Bx)	pH	Titratable Acidity (TA) (g/l)	Malic Acid (g/l)	FAN (mg/l)	Ammonia (mg/l)	Glucose (mg/l)	Fructose (mg/l)
2014/15	18.494	2.9095	15.939	8.978	171.87	101.45	93.969	81.039
2015/16	16.557	2.8039	15.498	7.287	91.04	115.01	81.053	74.217
P – values for the significance of 2014/15 versus 2015/16 seasons								
	< 0.001	< 0.001	<0.062	< 0.001	< 0.001	0.001	< 0.001	< 0.001

*FAN = Free alpha-amino nitrogen

4.5 Discussion

4.5.1 Effect of wētā damage on the yield of grapevines

The yield of grapevines has a number of different components. These are buds per vine, shoots per bud, clusters per shoot, berries per cluster and the weight of individual berries (Dry, 2000; Keller, 2010a). Wētā damage to buds at budburst affected each of these yield components, except the number of shoots/bud. This was unaffected because secondary shoots arose and replaced the primary ones after wētā had damaged most of the primary buds in the control and straw mulch treatments. However, these secondary shoots were relatively less productive than the primary ones, and their clusters, bunch number and bunch weights are smaller (Creasy & Creasy, 2009; Dry, 2000). In contrast, the efficacy of under-vine beans, sleeves and shell treatments at reducing damage to primary buds resulted in higher numbers of primary shoots in these treatments. Consequently, the yield in the latter treatments was higher than that in the control and straw mulch treatments.

The yield of Sauvignon Blanc increases linearly with the number of clusters per vine up to the point where the availability of assimilates becomes limiting. (Naor, Gal, & Bravdo, 2002). In this study, the number of clusters per vine in under-vine beans, sleeves and shell treatment probably exceeded this threshold. Hence, the lack of differences between the yields of vines in those treatments.

The differences between yield in the two seasons were partly due to weather patterns (Keller, 2010a; Khanduja & Balasubrahmanyam, 1972). The weather in a particular year determines the number of bunches per bud, or fruitfulness, in the following season (Dry, 2000; Vasconcelos, Greven, Winefield, Trought, & Raw, 2009). In contrast, bunch size (i.e. berry numbers and weight) is determined by the weather in the current season (Khanduja & Balasubrahmanyam, 1972; Sánchez & Dokoozlian, 2005; Sommer, Islam, & Clingeleffer, 2000; Vasconcelos et al., 2009). Both 2014/15 and 2015/16 had good weather in their preceding season. However, temperature and light intensity

during spring and flowering, when bunch size is determined, were relatively higher for the 2015/2016 than in 2014/15 (http://www.wineresearch.org.nz/category/weather-data/awatere-valley, Accessed on 29 July, 2016). Thus, the relatively good weather at budburst and flowering in 2015/16 enhanced the yield in that season. Also, the number of buds laid down in 2015/16 was higher than in 2014/15. During the latter season, there was a region-wide outbreak of powdery mildew (*Erysiphe necator* Schwein. (1834)) which further negatively affected yields. All of these factors contributed to the significant yield differences between the two seasons.

4.5.2 Efficacy of wētā management strategies

In the absence of appropriate management strategies, yield loss due to *H.* sp. 'promontorius', averaged over the two seasons, was *c.* 30.5%. The phenological stage (between budburst and the two-leaf stage) at which this insect damage vines is the similar to that of the rust mite, *Calepitrimerus vitis* (Nalepa). However, the highest loss due to the latter in vineyards is estimated at 23.7% (Walton et al., 2007). Other economically important vineyard pests such as leafrollers (Lepidoptera: Tortricidae) and mealybugs (*Planococcus* Migula 1894 spp.) (Hemiptera: Pseudococcidae) are reported to directly and/or indirectly cause up to 12% and 50% yield losses, respectively (Atallah et al., 2011; Lo & Murrell, 2000; Walton & Pringle, 2004). However, the latter pests can be managed with pesticides and/or biological control agents. These methods do not easily work with wētā and other similar orthopteran pests because of their nocturnal and subterranean behaviour (Musick, 1985).

To reduce this yield loss, the current work tested the effects of ground cover manipulation on this insect and its damage to vines. This strategy is often used for pest management in perennial crops (Fiedler, Landis, & Wratten, 2008; Zehnder, Gurr, Kühne, et al., 2007). Depending on the species of plant sown, it works by either serving as a trap plant for insect pests or providing resources (shelter, nectar, alternative food and pollen; SNAP) that increases the 'fitness' of natural enemies of pests. However, the natural enemies' advantage does not always lead to suppression of target pest species population (Berndt et al., 2002; Cook et al., 2006; English-Loeb, Rhainds, Martinson, & Ugine, 2003; Hassanali et al., 2008; Landis et al., 2000; Midega et al., 2008; Paredes et al., 2015; Rea et al., 2002; Rhino et al., 2014; Simpson et al., 2011; Villa et al., 2016). Here, tick beans sown under vines apparently served as alternative food for wētā, thus reducing their damage to vine buds at budburst. This strategy was effective because there were higher densities of this insect in the under-vine areas where the beans were sown (Nboyine et al., 2016).

In contrast, beans sown in the inter-rows were ineffective at preventing damage. This could be due to low wētā density in those areas (Nboyine et al., 2016). Since wētā densities are higher under vines than in the inter-rows, the insects had more frequent contacts with vines than bean plants in the IRTB treatment. This resulted in the vine buds sustaining significant damage in spite of the availability of alternative food in the inter-rows. However, feeding on beans in IRTB treatment increased slightly when access to the vines by wētā was denied by either tying the vine trunks with sleeves or spreading shells under vines.

Tick beans can be host to a range of arthropod herbivores at different growth stages. Some of the key insect pests at the vegetative stage include aphids [*Aphis fabae* Scopoli (Europe), *A. cracivora* Koch (Africa, America, and Australia), *Acrythosiphon pisum* Harris (worldwide)], thrips [*Thrips* spp. (worldwide)], budworms [*Helicoverpa armigera* (Hübner) (Australia, Eurasia, and Africa)], whitefly [*Bemisia tabaci* (Genn.) (Africa)], grasshoppers [*Chortophaga australion* Rehn & Hebard, *Microcentrum rhombifolium* (Saussure) (America)] etc. (Nuessly et al., 2004; Stoddard et al., 2010). However, apart from the grasshoppers, the other pest are not potential grape pests. Their threat can be minimised by removing the bean plants from vineyards after budburst; later vine growth stages are not damaged by wētā. Apart from pests, tick bean is also host to as many as 27 natural enemies of insect pests in the absence of insecticide applications in Florida (Nuessly et al., 2004). Some of these (especially the generalist predators) could contribute towards controlling the population of important vine pests such as the leafroller complex, mites etc.

Mulching the understoreys of vineyards or growing some plant species there can be an effective strategy for weed control, moisture retention and insect pest and disease reduction (Guerra & Steenwerth, 2012; Jacometti, Wratten, & Walter, 2007a, 2007b; Steinmaus et al., 2008; Thomson & Hoffmann, 2007). In the current work, mussel shell mulch halved the density of wētā. The shells appeared to be a physical barrier to wētā exiting their burrows at night. This is the first study of the effect of shell mulch on a soil burrowing orthopteran insect. However, Crawford (2007) reported a similar decrease in the abundance of earthworms in vineyards mulched with mussel shells. The worms were thought to abandon areas with the shells because of the reduction in availability of organic matter on the soil surface and/or their inability to occasionally reach the soil surface due to the shells. Here, this reduction in wētā density resulted in *c.*39% increase in grape yield compared to the control. In contrast to the present work, previous studies with mussel shells and other reflective mulches did not associate them with increased yield (Crawford, 2007; Creasy et al., 2007; Sandler, Brock, & Heuvel, 2009).

The straw mulch did not reduce wētā density and damage to vine buds. Mulch materials of plant origin can increase the assemblage of arthropod predators and microbial biocontrol agents, which in turn can reduce the numbers of insect pests (Addison et al., 2013; Thomson & Hoffmann, 2007). This did not occur probably because there is no known arthropod predator for this insect. A similar study by Gill, McSorley, and Branham (2011) also found that organic mulches had no effect on the abundance of orthopterans (Acrididae and Gryllidae) and that they were unaffected by the predator assemblage. Thus, damage by soil-dwelling orthopteran pests may not be effectively reduced with mulches of plant origin because there may be no relevant natural enemies of this group of insects or that the mulches do not serve as an effective barrier to the exit of these insects from the soil.

The three management approaches - under-vine tick beans, shell mulch and sleeve treatments - reduced wētā damage substantially and there were no significant differences between them in terms of vine yield components. The beans were less expensive (i.e., NZ$ 1.24/ kg and NZ$ 103.00/ ha for the entire under-vine area) and can easily be sown with planters modified for under-vine seed sowing. In addition to increasing natural enemy assemblages that could potentially reduce the population of other vine pests, they improve soil nitrogen content and condition (Köpke & Nemecek 2010). In contrast, mussel shells were freely available, but the cost of transporting them to vineyards was about NZ$ 18.00/ m^3. Accurate estimates of the transport cost of shells is difficult because it varies with distance between collection site and vineyard. However, this cost could be substantially reduced if they are transported in large trucks that can carry at least 10 tonnes of shells at a time. Shell mulches are applied once and they last for at least 5 years (Joanne Brady, Constellations Brand, pers. com.). Machines are also available for spreading the shells under vines. The sleeve treatment costs NZ$ 430.00 per ha, excluding the cost of repairing them annually. These sleeves have no additional beneficial role in vineyards, apart from mitigating wētā damage. Meanwhile, they litter vineyards when strong wind and/or grazing sheep remove them from vine trunks, thus polluting the environment. Hence, apart from the monetary cost, the labour needed to repair sleeves or re-plant beans annually, makes the use of shell advantageous even if initial cost is higher than any of the former. Furthermore, the negative consequences of plastics on the environment make bean treatment a better option because it provides other important ecosystem services, while mitigating wētā damage.

The significant difference in mean wētā density between the two seasons was mainly a site effect. Generally, the site used for the 2015/16 trial had higher densities of this insect than that used for the 2014/15. However, the efficacies of the management strategies tested were unaffected by these differences in density.

4.5.3 Maturity indices

Knowledge of grape maturity indices is of cardinal importance because wine quality is directly related to the quality of the vintage (Du Plessis, 1984; Ellis, Van Rooyen, & Du Plessis, 1985). These indices are mainly affected by environmental and management practices such as climate (temperature, rainfall and irrigation, light intensity and cloud cover etc.), soil (nutrition, terroir and plant density), yield and pruning and training system (Jackson & Lombard, 1993; Tesic, Woolley, Hewett, & Martin, 2002). The treatments tested here had no effect on any of these factors or practices, hence the lack of differences between treatments for the indices measured. The observed differences between these indices in the two seasons was probably due to climatic, soil and yiel d factors (Tesic et al., 2002). For instance, brix is low when temperatures are above 30 ° C or below 9 ° C and high when it ranges between 16 and 30 °C during growth stages I – III, while TA is high when night temperatures are below 15 ° C and low at > 15 ° C during stage III of growth. Similarly, brix is high when soil moisture is low (at stage III) and crop load is moderate (i.e., 4 – 10 kg/kg yield to pruning weight). In contrast, high moisture (> 150 mm rain) and crop load (> 10 kg/kg) reduces brix (Jackson & Lombard, 1993; Keller, Smithyman, & Mills, 2008). Thus, differences in climatic conditions and sites for the trial in 2014/15 and 2015/16 as well as the high yields in the latter season probably contributed to most of the maturity indices being lower in the 2015/16 season.

Conclusions

The use of pesticides to manage soil-dwelling insect pests is less effective than for other pest guilds and can result in outbreaks of secondary pests and leave residues in food. This work, therefore shows how simple locally available and inexpensive materials can be deployed to reduce damage by this group of insect pests in perennials such as vines. Mussel shell mulch was the best strategy to reduce wētā damage to vines. They appeared to be a good physical barrier to the insects exiting their burrow. Perhaps, other locally available dense materials, such as bark, could be used in perennial crops to reduce exit and/or emergence of soil-dwelling stages of arthropod pests at locations where mussel shells are unavailable or expensive. Tick beans sown under vines were also effective at reducing damage to vines by serving as alternative food to the insect. The treatments had no effect on grape maturity. However, further studies to develop protocols on the number of vine rows that should be mulched with mussel shells or sown with under-vine tick beans per hectare are needed.

Chapter 5

Deterring wētā from vineyards

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5.1 Abstract

The effects of the association between grasses and fungal endophytes on orthopterans are very poorly studied although they are important pests. Here, the endemic New Zealand wētā, *Hemiandrus* sp. 'promontorius', and *Festulolium loliaceum* infected with *Epichloë uncinata,* were used to study the effect of endophyte-mediated resistance in grasses on this large orthopteran insect in the laboratory, and the possibility of using endophyte-infected grasses to 'push' wētā out of vineyards. Wētā were presented with *F. loliaceum* with and without *E. uncinata* infection in no-choice and paired choice experiments. Other controls were *Epichloë festucae* infected *Festuca rubra* and endophyte-free *Lolium perenne.* The endophyte-infected grasses, Barrier U2 (*Festulolium loliaceum*), Easton MaxP (*Festuca arundinacea*) ad Matrix SE (*L. perenne*), were also tested for their efficacy to repel wētā from vineyards when sown as inter-row ground cover. The current wētā management strategy of tying plastic sleeves around vine trunks and vines without the sleeves were included as control treatments for the field trials. In the laboratory no-choice experiments, persistent attempts by the insect to graze the endophyte-infected grasses (but promptly abandoning them) resulted in a significantly higher number of plants lost due to excision at their stems after the first bite ($P = 0.004$). The inability of affected grasses to compensate for the lost biomass resulted in a lack of significant difference between the dry biomass of endophyte-infected and endophyte-free controls ($P = 0.206$). However, in choice experiments, there was a preference for the endophyte-free controls when they were paired with the endophyte-infected grasses ($P < 0.05$). Results of the field studies showed significant reductions in wētā densities in some endophyte-infected grass treatments at one of the trial sites. However, it was only in Barrier U2 that this reduction in density resulted in significant grape yield increment. The current work therefore suggests that endophyte-infected grasses maybe used to 'push' wētā out of vineyards; but further tests are needed.

Key words: *Epichloë uncinata,* loline alkaloids, *Festulolium loliaceum,* biomass loss, Orthoptera, pest management

5.2 Introduction

Crop losses due to pests (weeds, insects and pathogens) are estimated to range from 50 to 80% globally and insect pests account for about 18% of this loss (Oerke, 2006). Conventional farming practices using pesticides can contribute to mitigating losses by insect pests but are not sustainable (Godfray & Garnett, 2014). Apart from killing the target insect pests, insecticides also generate external costs such as killing pollinators and pests' natural enemies (Fernandes, Bacci, & Fernandes, 2010; Jones et al., 2014) as well as those associated with the applications themselves, such as the agro-chemical, fuel and capital depreciation (Haverkort et al., 2008). It follows that achieving food security while averting the negative consequences of conventional approaches to crop production requires the adoption of sustainable agricultural practices for the protection of crops from pests (Poppy, Jepson, Pickett, & Birkett, 2014; Shennan, 2008).

These sustainable practices include the exploitation and enhancement of a plant's ability to defend itself from insect pest attack (Kumari, Reddy, & Sharma, 2006; Mortensen, 2013; Ronald, 2011). For instance, some plants produce constitutive (i.e., always present) specialised bioactive compounds (alkaloids, cyanogenic glucosides, glucosinolates, phenolics, terpenoids etc.) that defend them against insects (Fürstenberg-Hägg, Zagrobelny, & Bak, 2013; War et al., 2012). These specialized compounds may act by having adverse physiological effects on the insect after ingestion of the plant (i.e., antibiosis) or by deterring feeding and/or oviposition by insects (i.e., antixenosis) (Fürstenberg-Hägg et al., 2013; Kogan, 1994; War et al., 2012). Certain morphological features (e.g., trichomes, epicuticular waxes etc) are also constitutive and may be involved in antixenosis (Fürstenberg-Hägg et al., 2013; Kogan, 1994). Transgenic maize, cotton and other such crops use antibiosis as their defence mechanism (Brévault et al., 2013) while some genotypes of pigeonpea use antixenosis as a defence against feeding damage and oviposition by the lepidopteran pest *Helicoverpa armigera* (Hübner, 1809) (Kumari et al., 2006). Similar defences can be induced in response to insect feeding or even the release of insect pheromones (Helms, De Moraes, Tooker, & Mescher, 2013).

Other plants, especially grasses, form symbiotic associations with certain fungi (i.e., endophytes) which protect them from herbivores. These produce a range of toxic alkaloids, including peramine, ergot alkaloids, lolitreme and loline, which have anti-insect and/or anti-vertebrate effects (Azevedo, Maccheroni Jr, Pereira, & de Araújo, 2000; Guerre, 2015). Lolitreme and ergot alkaloids are toxic to insects and vertebrates while peramine and loline alkaloids affect insects only (Azevedo et al., 2000; Popay & Hume, 2011). These toxins are constitutive but induction also occurs in response to herbivory (Patchett, Chapman, Fletcher, & Gooneratne, 2008).

Over the last two decades, many endophyte-infected grass cultivars that possess anti-insect but not anti-vertebrate alkaloids have been bred from species of *Festulolium* Asch., *Festuca* Linn. and *Lolium* Linn. for enhanced pasture production (Fletcher, 1999; Patchett, Gooneratne, Chapman, & Fletcher, 2011; Popay & Hume, 2011). These grasses are infected with strains of endophyte species from the genus *Epichloë* (Faeth & Saikkonen, 2007; Guerre, 2015; Schardl et al., 2013). Their bio-pesticidal effect on insect pests depend on the spectrum and concentration of the alkaloids they produce. Thus, the benefits from these grasses can be optimized by choosing those that contain the endophyte appropriate to the target pests (Popay & Hume, 2011). This is because the outcome of an endophyte-host grass-insect pest interaction depends on the grass species or genotype, the endophyte type, and the feeding behaviour of the insect species involved (Afkhami & Rudgers, 2009; Ball & Tapper, 1999; Clement, Elberson, Bosque-Pérez, & Schotzko, 2005; Faeth & Saikkonen, 2007).

Epichloë uncinata U2-infected *Festulolium loliaceum* (Huds.) P. Fourn. (*Festuca pratensis* Huds. × *Lolium perenne* Linn.) is an example of an endophyte-infected grass with bio-pesticidal effects on insects. This fungus produces loline alkaloids which deter feeding by the major pests in Australasian pastures: grass grub (*Costelytra zealandica* (White, 1846)), black beetle (*Heteronychus arator* (Fabricius, 1775)) black field cricket (*Teleogryllus commodus* (Walker, 1869)), *Lepidogryllus* sp. and wingless grasshopper (*Phaulacridium vittatum* (Walker, 1870)) (Barker, Patchett, & Cameron, 2015a; Barker, Patchett, & Cameron, 2015b; Patchett et al., 2011). The efficacy of this endophyte strain in *F. loliaceum* against other large, occasional orthopteran grassland pests is not known. Even the effects of *Epichloë* infection in other grass hosts on grazing by grasshoppers are so far inconclusive (Afkhami & Rudgers, 2009; Lewis, White, & Bonnefont, 1993; Lopez, Faeth, & Miller, 1995; Zhang, Li, Nan, & Matthew, 2012). Meanwhile, the occasional outbreaks of these insects result in significant yield losses in the absence of appropriate plant protection measures (Branson, Joern, & Sword, 2006).

Here, the endemic New Zealand ground wētā, *Hemiandrus* sp. 'promontorius' (Johns, 2001) (Orthoptera: Anostostomatidae), and *E. uncinate*-infected *F. loliaceum* were used to study the effects of endophyte-mediated resistance in grasses on large orthopterans and potential of such grasses deterring wētā from vineyards when used as inter-row vegetation.

5.3 Materials and methods

5.3.1 Laboratory experiments

5.3.1.1 Study grasses and laboratory conditions

No-choice and choice experiments were used to test the resistance of *E. uncinata* U2-infected *F. loliaceum* to the insect, *H.* sp. 'promontorius'. An *E. festucae*-infected *Festuca rubra* (also known to be resistant to insect herbivores) and endophyte-free *L. perenne* and *F. loliaceum* were included as controls in the study. The inclusion of *L. perenne* and *F. rubra* was to examine the robustness of the pattern of insect behaviour and plant response across different grass taxa and endophytes. A summary of the characteristics of the grasses used is given in Table 5.1.

Seeds of these grasses were planted in 300ml plastic pots filled with sandy loam soil in a glasshouse at Lincoln University, Christchurch, New Zealand (Fig. 5.1). Three weeks after germination, the grasses were thinned to 50-60 plants per pot before being used for the laboratory bioassays. This seedling density is not unusual in laboratory experiments of this type (Barker et al., 2015a; Barker et al., 2015b).

Table 5.1 Key characteristics of grasses used

Scientific name	Common/ commercial name	Endophyte present	Endophyte	Toxins produced
Festulolium loliaceum (*Festuca pratensis* × *Lolium perenne*)	Barrier U2	Yes	*Epichloë uncinata*	Loline alkaloids
Festuca rubra	Fine fescue	Yes	*Epichloë festucae*	Ergovaline, Lolitreme B
Festulolium loliaceum	Barrier Nil	No	-	
Lolium perenne	Ruanui	No	-	

Figure 5.1 Grass treatments at the nursery before experiments

Figure 5.2 Plastic arenas arranged in a randomized block design in the Controlled temperature (CT) room

The bioassays were conducted in a controlled temperature (CT) room from 19 February to 2 July, 2015 at the Bio-Protection Research Centre, Lincoln University, New Zealand (Fig. 5.2). The temperature in the room was 20 °C with a 4 °C range and 16 h daylength to mimic the field conditions under which *H.* sp. 'promontorius' feeds (Johns, 2001).

5.3.1.2 No-choice experiment

A randomized complete block design with six replicates per treatment was used for these feeding tests. The treatments comprised the four grasses listed in Table 5.1. Wētā fed on the usual laboratory diet of organically grown carrots were included to check that background feeding rates were normal. The grass treatments without the test insect were also included as checks to measure the effects wētā feeding activities on the biomass of the grasses.

Plastic arenas (17 mm ×17 mm × 19 mm) were filled to half their volumes with sandy loam soil collected from an organic farm at Lincoln University, Christchurch. Soil from this site was used because it is free from pesticide residues that may have adverse effects on this burrowing insect and the results of the experiments. The soil in the centre of each arena was scooped out and a plastic pot which contained the test grass was placed in the depression created. The surface of the soil in the arenas were levelled to cover gaps (Fig. 5.3). A single pre-weighed unsexed mid instar wētā nymph was introduced into each of the arenas, the tops of which were covered with perforated lids. The bioassays were assessed at 7 and 14 days after adding the insect.

Figure 5.3 A plastic showing placement of grass treatment in a no-choice experiment

5.3.1.3 Choice experiment

Paired choice experiments were used to assess the preference of this insect for either the endophyte (E+) or non-endophyte (E-) infected grasses. The treatment pairs were (see Table 5.1):

1. Barrier U2 versus Barrier Nil,
2. Barrier U2 versus Ruanui,
3. Fine fescue versus Barrier Nil, and
4. Fine fescue versus Ruanui.

These treatments have a 2 × 2 factorial structure.

Two plastic pots (each containing 50 individual plants of one grass treatment) were placed opposite each other in arenas pre-filled to half their capacity with sandy loam soil as described above (Fig 5.4). One pre-weighed unsexed wētā was placed between the pair of grass treatments in each of the

arenas. Preliminary and final assessments of the experiments were conducted 7 and 14 days after the test insect was introduced into the arenas.

There were five replicates (arenas) of each of the four treatment pairs and these were arranged in a randomized complete block design. Different shelves in the same CT room served as blocks. All four treatment pairs without the test insect were also present in all five replicates. Overall, there were 8 = 2 x 2 x 2 treatment pairs, each in one of eight arenas that were randomised in each block.

Figure 5.4 Fescue (left) and ryegrass (right) treatment placed opposite each other in a plastic arena. (Note: picture was taken before thinning to 50 plants)

5.3.1.4 Data collection for laboratory experiments

1. Weight change of wētā

The weight of wētā before and after the trial was measured and the ratio of final to initial weight was calculated.

2. Wētā survival

Wētā that survived in each treatment were assigned a score of 1 and those that died were scored as 0.

3. Damage scores

Wētā feeding damage to the grasses was scored on a scale of 0 to 10, with 0: no feeding; 1: 1 - 10% of plants damaged; 2: 11 – 20%; … 10: 91 - 100%.

4. Severed plants

The number of plant stems that were severed by wētā were counted. 'Severed plants' means all plants excised at the base of the stems but not consumed by wētā.

5. Plant biomass

At the end of the feeding trial, the fresh plants (including the severed pieces) were washed thoroughly to remove all soil and weighed, after which loline alkaloid samples we re taken as below, then the remainder was oven dried at 65°C for 48 h and dry weight recorded.

6. Analysis of plants for loline alkaloids

Samples of each grass treatment (each > 500mg fresh weight excluding the roots) were washed and dried with paper towels. They were immediately frozen in liquid nitrogen, ground into fine powder and freeze dried. A method modified from Blankenship et al. (2001) was then used to analyse the loline alkaloid content of each sample. Briefly, the extraction involved passing 100 mg of each sample in 5 ml of dichloromethane: ethanol (95: 5) solvent containing 6mg phenylmorpholine/ 100 ml of solvent as the internal standard, along with 250 µl saturated sodium bicarbonate. They were then shaken at room temperature for 1 h at 200 rpm on an orbital shaker and left to settle for 10 mins before being filtered into 2 ml GC vials. A Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector was used to analyse the filtrates. Hydrogen passed through an Rtx-624 column was used as the carrier gas in these analyses. The retention times for *N*-methyl loline (NML), *N*-acetyl norline (NANL), *N*-formyl loline (NFL) and *N*-acetyl loline (NAL) were 12.8, 17.4, 18.2 and 18.8 minutes, respectively.

5.3.1.5 Data analyses for laboratory experiments

For both experiments, the weight changes of wētā were measured by subjecting the ratio of wētā weight after and before the trials to logarithmic transformation in order to ensure homogeneity of variances. The variable used in the statistical analysis was $\log_{10}$[(Final wētā weight) / (Initial wētā weight)]. In the no choice experiment, the relative effect of the wētā on the dry biomass of the grass was measured by calculating the variable $\log_{10}$[(Dry biomass in presence of wētā) / (Dry biomass in absence of wētā)] in each block using the corresponding pairs of arenas. In the choice experiment,

the relative effect of the presence or absence of endophyte (E+ versus E-), in the presence of wētā as compared to the absence of wētā, on the dry biomass was measured by calculating, for each treatment pair, the variable;

$$\log 10\left[\frac{\text{Dry biomass of E}+\text{ grass in presence of wētā}\,/\,\text{Dry biomass of E}-\text{ grass in presence of wētā}}{\text{Dry biomass of E}+\text{ grass in absence of wētā}\,/\,\text{Dry biomass of E}-\text{ grass in absence of wētā}}\right]$$

This was calculated for each block using data from the corresponding pairs of arenas. It is known as a Before-After-Control-Impact (BACI) variable (McDonald, Erickson, & McDonald, 2000), and is designed to compare the relative biomasses of E+ and E- in the presence of wētā, after adjustment for their relative biomasses in the absence of wētā. The resulting data were subjected to an analysis of variance using GenStat® version 16 Statistical Package. Means were separated using an unrestricted least significance difference procedure (LSD) at $P < 0.05$. In the choice test, significant differences in the extent of feeding damage between the grass treatments offered to wētā were detected by computing the mean difference between the damage score for each pair and testing its significance against zero with the least significant effect (LSE) at 5% level (this was calculated from the LSD by dividing the latter by √2). Differences in the extent of plant excision and dry biomass loss between grass pairs were also determined by calculating the mean differences and using the LSE to test their significance against zero. A two-sample 2-sided *t* test was used to test the null hypothesis H_0: mean concentration of loline alkaloids in Barrier U2 with wētā feeding damage did not differ significantly from those in Barrier U2 without wētā feeding damage.

5.3.2 Study sites for field experiments

The study was conducted in the Awatere Valley, Marlborough at two sites (N-block and R-block) in vineyards belonging to Constellation Brands NZ. The vine cultivar was Sauvignon Blanc. They were planted at a spacing of 2.4 m × 1.8 m (inter-vine × intra-vine).

5.3.2.1 Experimental layout

The endophyte-infected grasses, Barrier U2, Easton MaxP (Fescue: *Festuca arundinacea* Schreb.) and Matrix SE (Ryegrass: *Lolium perenne*), were tested for their efficacy to repel wētā from vineyards when sown as ground cover in the inter-rows. The current wētā management strategy of tying plastic sleeves around vine trunks and vines without the sleeves were included as control treatments. The inter-rows in the latter treatments contained the existing vegetation dominated by endophyte-free ryegrass. Thus, there were five treatments and these were replicated five times in each trial site.

These treatments were arranged in a linear randomized complete block design. The grass treatments were sown at a rate of 20 kg/ha and to a soil depth of 1 – 2 cm in each treatment plot. 'Plot' refers to the four vines in a bay and its two inter-rows. 'Block' consisted of the five treatment plots in each vine row. Figure 5.5 shows the experimental layout in each site.

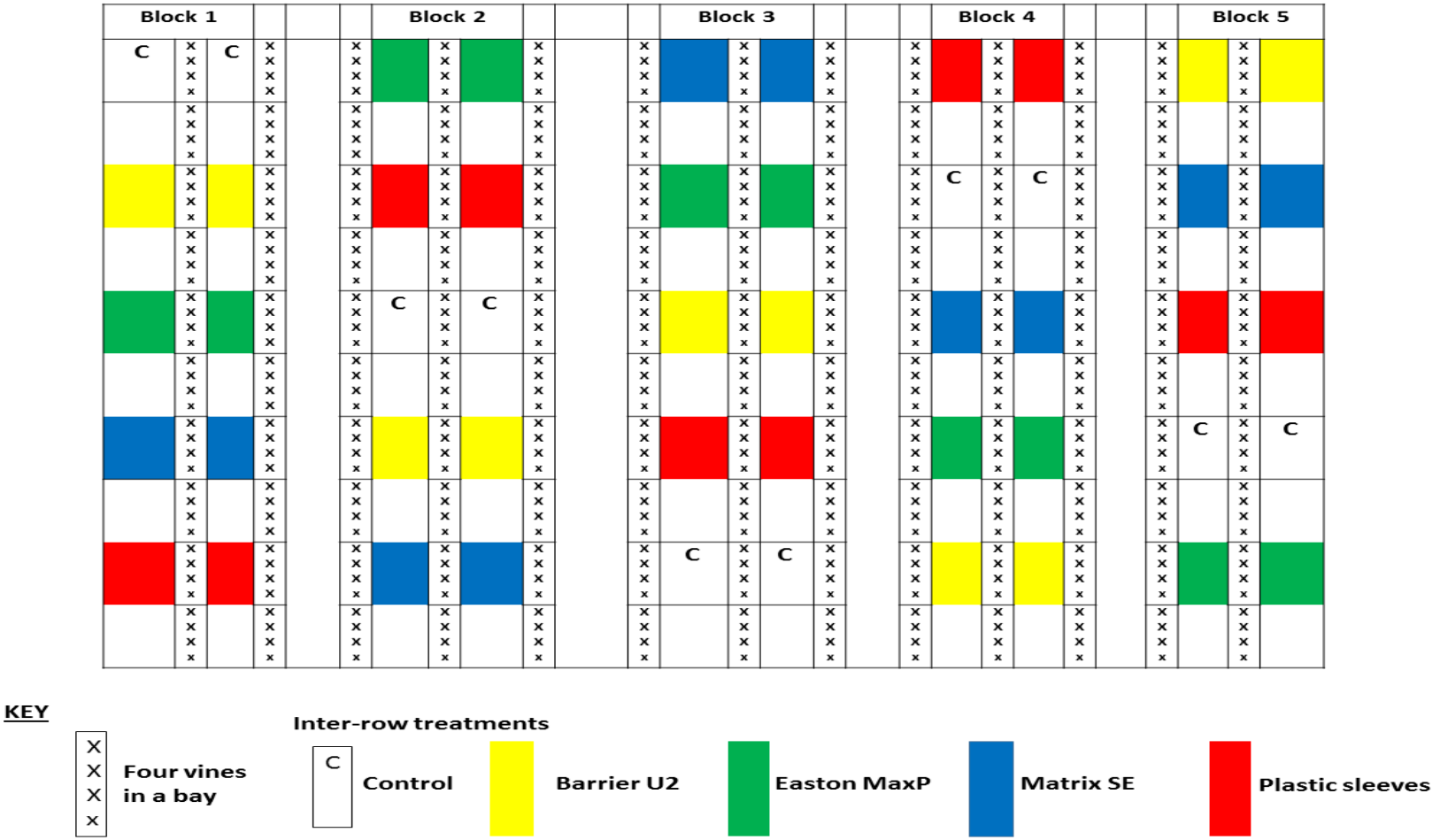

Figure 5.5 Experimental layout in the vineyards in the 2015/16 season for field trial with endophyte-infected grasses.

5.3.2.2 Data collection

Data on components of grapevine yield and yield were collected from the two middle vines for all treatments. These were number of buds laid down, number of shoots/bud, number of clusters/shoots, bunch weight, number of bunches and grape yield.

The initial wētā density was assessed in the area between the two middle vines of bays opposite treatment plots to avoid disturbing those residing in actual treatment plots. This was done by scraping off the top soil to expose burrows present and counting them. Three burrows were randomly chosen and excavated to confirm the presence of the insect. Density was therefore, estimated as the proportion of burrows with wētā per unit area. This estimate was assumed to be the same as that in the experimental plot because earlier studies had found under-vine density of this insect not to differ significantly. The final density was estimated in the actual treatment plots at the end of the trial.

5.3.2.3 Analyses of field data

A general analysis of variance was performed on all variables measured. Means that were significantly different were separated using the least significant difference at 5% probability level. For data on wētā density, the logarithmic ratio of final to initial wētā density was computed before subjecting the resulting data to ANOVA. The resulting ratio is called change in density.

5.4 Results

5.4.1 No-choice experiment

There was a significant difference in the rate of feeding damage sustained by the different grasses ($P < 0.001$; Table 5.2). Fine fescue and Barrier U2 (both endophyte-infected) had the lowest damage, significantly less than both Ruanui and Barrier Nil (both endophyte-free) which had the highest damage ($P < 0.001$). Damage was not significantly different between Barrier U2 and fine fescue. Similarly, there was no significant difference between the rate of feeding damage sustained by Barrier Nil and Ruanui.

The number of plants severed at their stem bases but not consumed by the insect was significantly different among the grass treatments ($P = 0.004$; Table 5.2). Fine fescue and Barrier U2 had the highest number of plants severed in this way, significantly higher than both Barrier Nil and Ruanui ($P < 0.001$). There was no significant difference between the number of severed Barrier U2 and fine fescue plants. The lowest numbers of severed plants were found in Barrier Nil and this was not significantly different from that of Ruanui.

Most severed plants died in all treatments leaving biomass on the soil surface for subsequent weighing, along with the few remaining intact plants ($P = 0.206$; Table 5.2). When the dry biomass was compared between wētā and non-wētā infested plants for each grass treatment, there were no significant differences among the four grasses ($P = 0.703$) (Table 5.2). Wētā lost weight by an average of 4 – 8% (Table 5.2). The ratios of final to initial wētā weight, and survivorship of wētā exposed to the different grass treatments were not significantly different among the treatments.

Table 5.2 For the no choice experiment, effect of the feeding activities of wētā on the damage score and number of severed stems of the grass treatments tested, and weight change and survivorship of wētā exposed to the different treatments. For biomass, the ratio between grass treatments in the presence and absence of wētā is presented.

Grasses	Feeding damage score	Number of severed stems	Log_{10} ratio of plant dry biomass [wētā / (no wētā)]		Log_{10} ratio of wētā weights (final / initial)		Wētā survivorship (proportion)
Barrier U2	2.2	19.7	0.309	(2.0)	-0.016	(0.96)	1.00
Fine fescue	1.5	20.0	0.258	(1.8)	-0.035	(0.92)	0.67
Barrier nil	7.5	3.5	0.375	(2.4)	-0.024	(0.95)	1.00
Ruanui	8.3	7.3	-0.156	(0.7)	-0.028	(0.94)	0.67
Overall P value	<0.001	0.004	0.703		0.979		
LSD (5%)	2.1	9.7	1.089		0.099		
Significance of endophyte-infected versus endophyte-free:							
P value	<0.001	<0.001	0.626		0.979		

For the fourth and fifth columns, back-transformed means are given in brackets.

Ruanui, Barrier Nil and fine fescue did not contain loline alkaloids. However, the fine fescue contained lolitreme B and ergovaline but the concentration of this toxin was not analysed. After insect wounding, Barrier U2 had high concentrations of the alkaloids, *N*-acetyl loline (NAL), *N*-acetyl norloline (NANL), *N*-formyl loline (NFL) and *N*-methl loline (NML) but these were not significantly different from those in the controls without feeding wounds. The mean NFL concentration was the highest while that of NML was the lowest. Overall, the total loline concentration increased in grasses exposed to wētā although this was also not significantly different from that of those not exposed to insect (Table 5.3).

Table 5.3 Concentration (µg/g) of loline alkaloids in *E. uncinata* U2-infected *F. loliaceum* (Barrier U2) in the presence and absence of wētā in a no-choice test (n = 5)

Loline alkaloids	Concentration (µg/g)		*t* value (2 tailed)	*P* value
	Wētā present	Wētā absent		
N-acetyl loline (NAL)	779	639	0.31	0.761
N-acetyl norloline (NANL)	329	211	0.65	0.522
N-formyl loline (NFL)	2855	1752	0.91	0.372
N-methyl loline (NML)	107	21	1.39	0.192
Total	4070	2623	0.77	0.452

5.4.2 Choice experiment

Table 5.4 shows the effects of the insect's feeding on the grasses under choice conditions and the effect of the grasses on the weight change and survivorship of the insect. Wētā caused significantly higher damage to Barrier Nil than either of Barrier U2 or fine fescue (since the first two mean differences in damage score in Table 5.4 are both greater than the LSE (5%) of 2.0). Ruanui was significantly more damaged than either Barrier U2 or fine fescue. Examination of the 2 x 2 factorial contrasts for differences in damage score revealed no significant main effect differences between the endophyte-infected (E+) grasses Barrier U2 and fine fescue nor between the endophyte-free (E-) grasses Barrier Nil and Ruanui (Table 5.4). There was also no significant interaction, with the difference between the preference of wētā for the E- grasses Barrier Nil and Ruanui being similar regardless of which E+ grass was present ($P = 0.297$).

The numbers of severed Barrier U2 and fine fescue plants were not significantly different from those of Barrier Nil and Ruanui in these choice tests, except that fine fescue had a significantly higher number of severed plants than Ruanui ryegrass (since the mean difference of 8.0 in Table 5.4 was higher than the LSE (5%) of 6.6).

The dry biomass of Barrier Nil in the presence of Barrier U2 or fine fescue was proportionately significantly lower after wētā feeding (compared to no wētā feeding) than either of the latter E+ grasses (since the first two log(BACI) means differed from 0 by more than the LSE (5%) of 0.499). Ruanui was also proportionately significantly lower in dry biomass than either Barrier U2 ($P < 0.10$) or fine fescue ($P < 0.001$) (in the presence as compared to the absence of wētā feeding). There was a 10% significant interaction between E+ grass and E- grass for this log(BACI) variable ($P = 0.052$). This interaction was caused by the marked difference in $\log_{10}$(BACI) mean between the Fescue – Ruanui treatment pair and the other three treatment pairs. For the Fescue – Ruanui treatment pair, the dry biomass of E+ fescue was 56 times higher than that of E- Ruanui in the presence of wētā (after adjustment for the ratio of remaining dry biomass in the absence of wētā).

In this experiment, there was no mortality of the wētā, and the wētā increased in weight by an average of about 7% (Table 5.4), with the increase being statistically significant in just one treatment, the Barrier U2 – Barrier Nil treatment. Their proportional weight change did not differ significantly among the treatments (Table 5.4), nor were any of the main effect or interaction contrasts significant.

Table 5.4 In the choice experiment, effect of wētā feeding preference for endophyte-infected (E+) and endophyte-free (E-) grasses on differences between E+ and E- in damage score and number of severed plants, and ratios of plant dry biomass (E+ / E- for wētā/(no wētā)) and weight of the wētā (final / initial). Note that all wētā survived in this experiment.

Choice pairs (Endophyte ([1]E+) + Non-endophyte ([2]E-)-infected grasses)	Mean difference of damage score (E- – E+)	Mean difference of number of severed plants (E+ - E-)	Log_{10} [3]BACI of plant dry biomass (E+ / E-)		Log_{10} Ratio of final wētā weight to initial	
Barrier U2 - Barrier nil	6.2	0.4	0.508	(3.2)	0.067	(1.17)
Fescue - Barrier nil	8.4	1.0	0.796	(6.3)	0.000	(1.00)
Barrier U2 - Ruanui	5.8	1.4	0.473	(3.0)	0.033	(1.08)
Fescue - Ruanui	6.0	8.0	1.749	(56.1)	0.011	(1.03)
P –values for 2 x 2 factorial:						
Main effects						
Endophyte (E+)	0.215	0.261	0.005		0.103	
Non-endophyte (E-)	0.153	0.214	0.068		0.662	
Interaction effect						
E+ × E-	0.297	0.345	0.052		0.398	
LSD (5%)	2.8	9.4	0.705		0.078	
[4]LSE (5%)	2.0	6.6	0.499		0.055	

[1]E+ = Endophyte infected grasses; [2]E- = Non-endophyte infected grasses; [3]BACI = (Dry biomass ratio, E+/E-, of grasses presented to wētā)/ (Dry biomass ratio, E+/E-, of grasses not presented to wētā); [4]LSE = Least Significant Effect, for comparing a mean with zero; For the fourth and fifth columns, back-transformed means are given in brackets.

In the choice tests, the concentrations of NAL, NANL and NFL were significantly higher in Barrier U2 exposed to wētā than Barrier U2 not exposed to wētā. There was no significant difference between the concentrations of NML in wētā-wounded and unwounded Barrier U2. As in the no-choice test, the alkaloid with the highest concentrations was NFL and the lowest was NML. The total loline concentration in Barrier U2 in the presence of wētā was approximately three times higher than in the absence of wētā (*P* = 0003; Table 5.5).

Table 5.5 Concentration (µg/g) of loline alkaloids in *E. uncinata* U2-infected *F. loliaceum* (Barrier U2) in the presence and absence of wētā in a choice test (n = 5).

Loline alkaloids	Concentration (µg/g)		*t* -value (2-tailed)	*P*- value
	Wētā present	Wētā absent		
N-acetyl loline (NAL)	1784	532	3.64	0.001
N-acetyl norloline (NANL)	485	181	2.88	0.008
N-formyl loline (NFL)	4394	1608	3.29	0.003
N-methyl loline (NML)	78	33	1.59	0.126
Total	6741	2354	3.39	0.003

5.4.3 Field results

Generally, there was poor establishment of the grass treatments in the N- block. As a result, there were no significant treatment effects for any of the variables measured. The combined analyses were affected by data from the N- block and did not also show a significant treatment effect for all variables at the 5% probability level. The results presented here therefore, focus on the R- block.

5.4.3.1 Effects of inter-row ground cover treatments on components of yield and yield of grapevines

The number of buds laid down per vine at the beginning of the experiment was not significantly different among the grass treatments in the R- block (P = 0.195; Fig. 5.6). The mean number of buds laid down in the R- blocks was 48 buds/vine. Similarly, the mean number of shoots/bud was not significantly different between treatments (P = 0.207; Fig. 5.7).

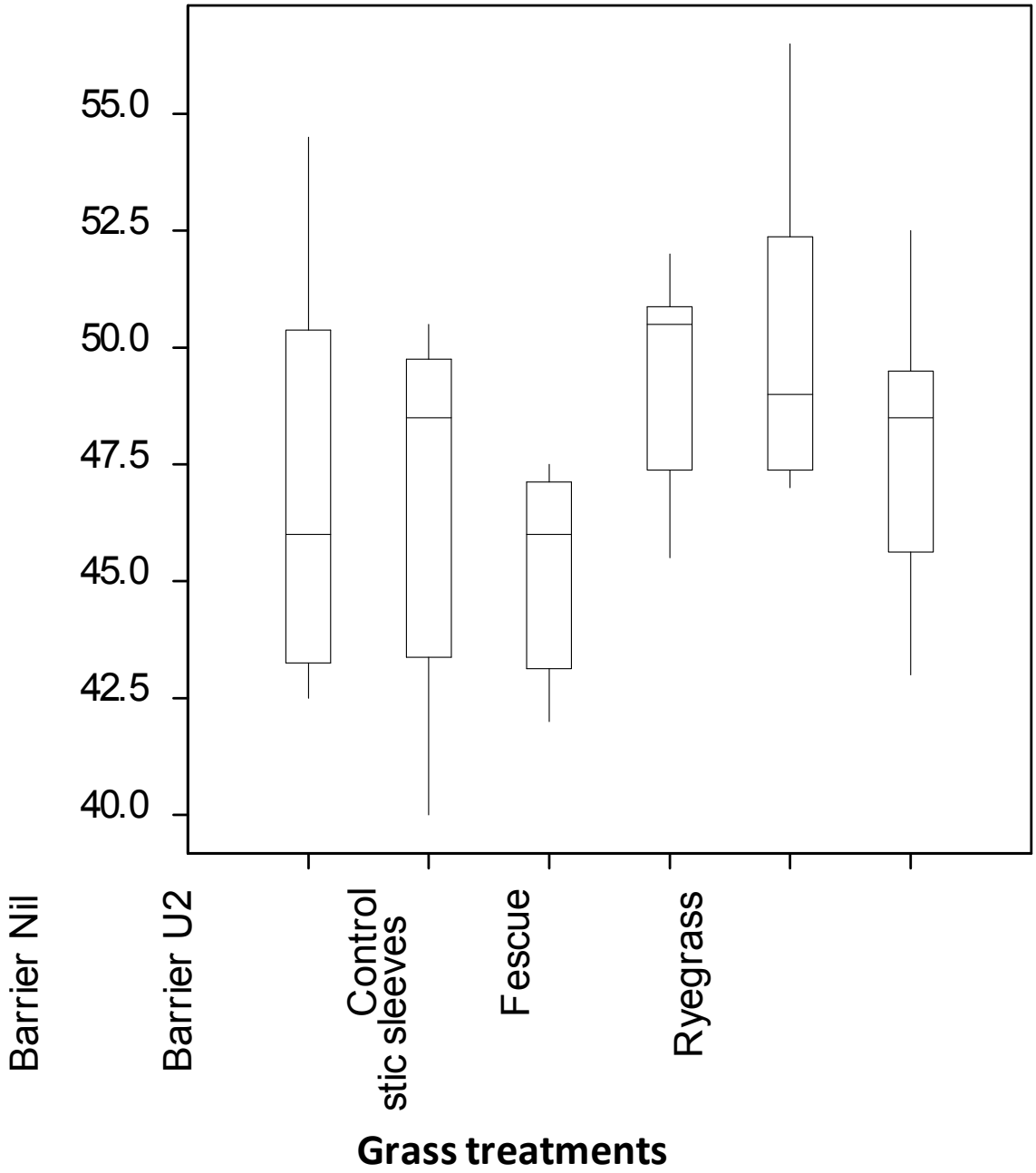

Figure 5.6 Boxplot showing number of buds laid down in the R-block.

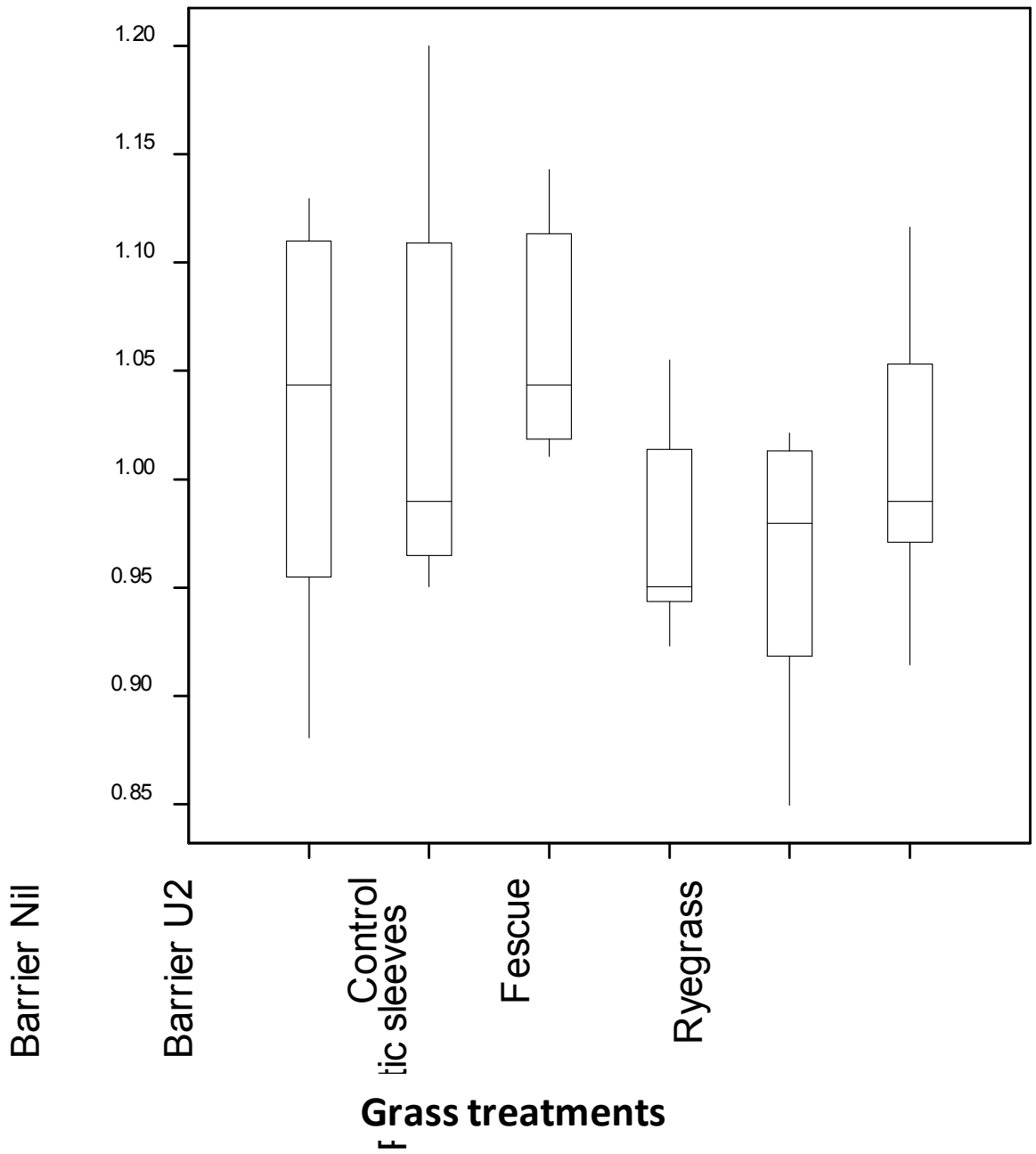

Figure 5.7 Boxplot showing the effect of ground cover treatments on the number of shoots / bud in the R-block.

However, there were significant differences between the grass treatments for the number of bunches/vine ($P < 0.001$). There was about 99% significant increases in the number of grape bunches in plastic sleeves protected vines compared to the control. There was no significant difference between number of bunches in sleeves and Matrix SE protected vines. The bunches in Barrier U2, Easton MaxP and control treatments were also not different from each other (Fig. 5.8).

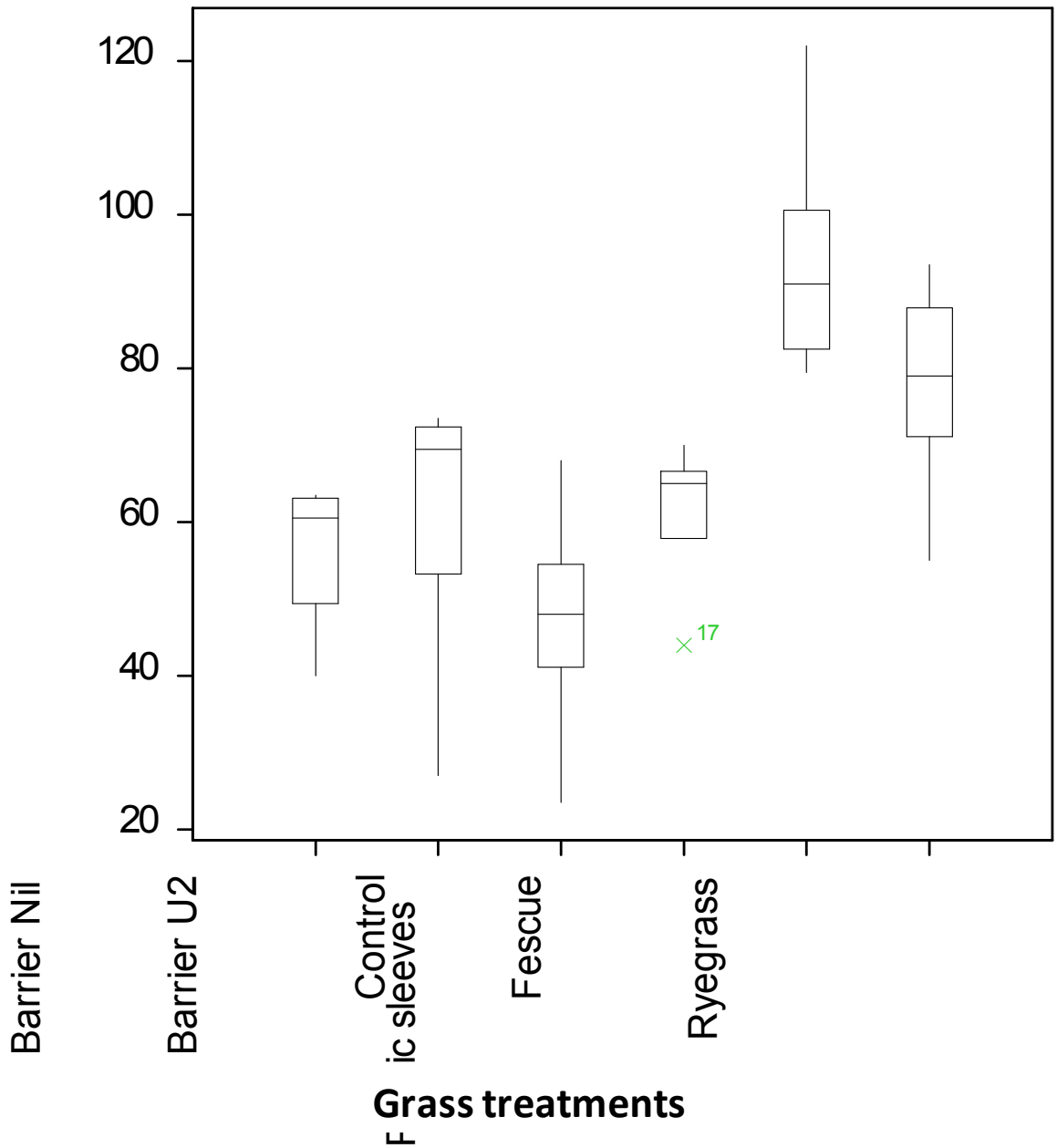

Figure 5.8 Boxplot showing the effect of ground cover treatments on the number of bunches/ vine in the R-block.

The weight of grape bunches was not significantly affected by the grass treatments (P = 0.099; Fig. 5.9).

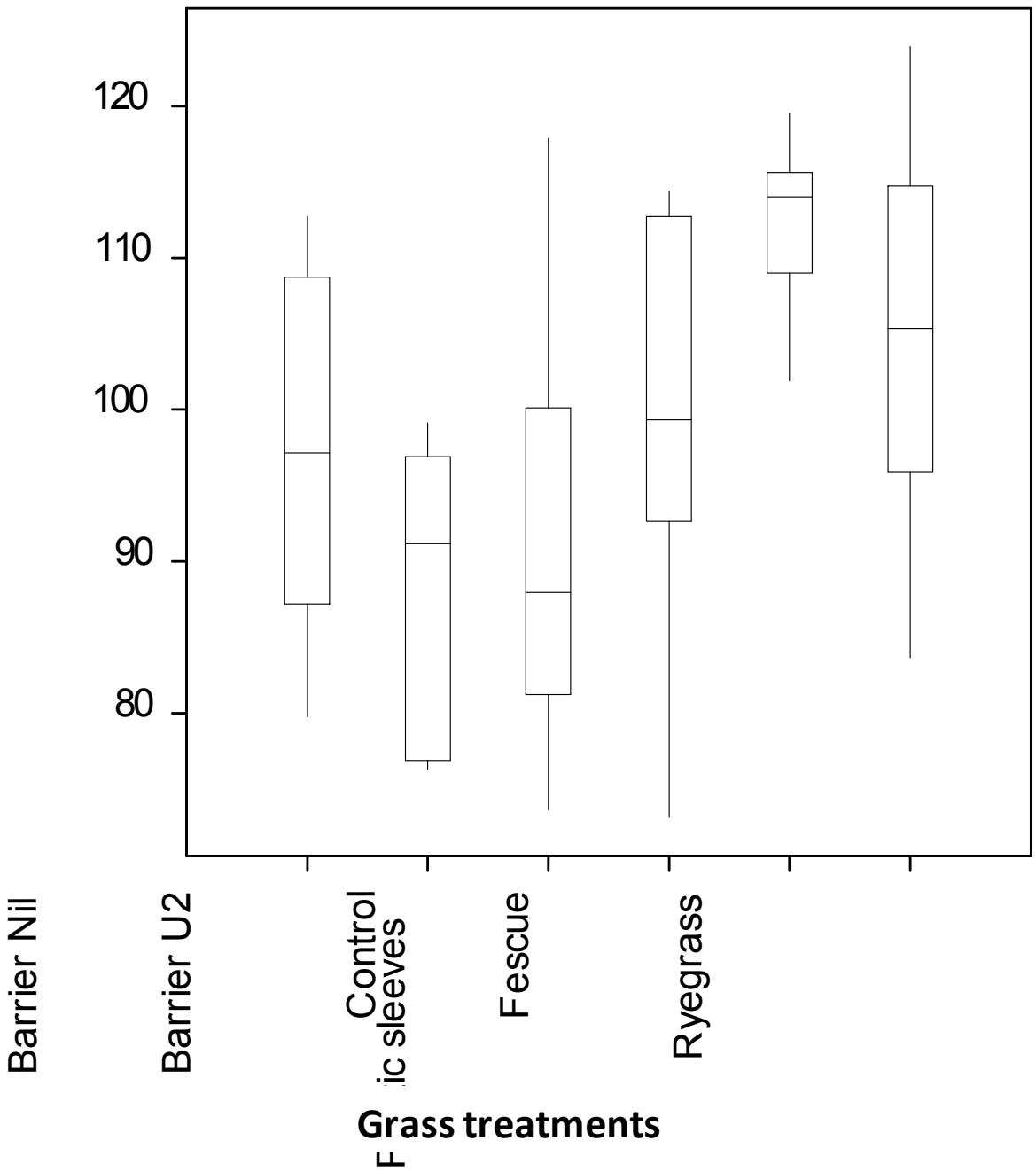

Figure 5.9 Boxplot showing the effect of ground cover treatments on the bunch weight (g) in the R-block

The grass treatments significantly increased the number of clusters/shoot (P = 0.002). Vines with plastic sleeves recorded the highest number of cluster/shoot, while the control was the lowest (Fig. 5.10). There were no significant differences between Barrier U2, Easton MaxP and the control treatments for this variable.

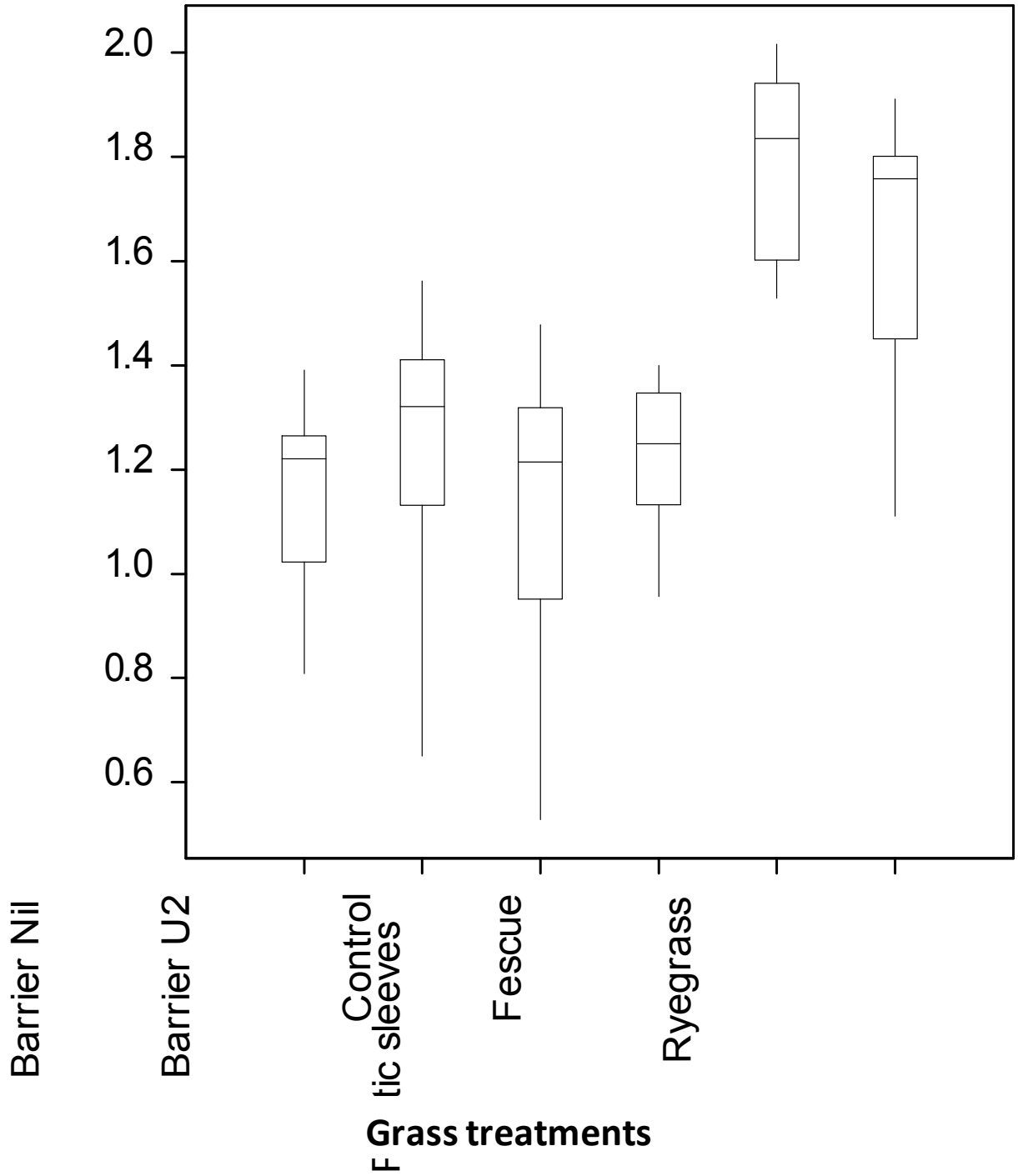

Figure 5.10 Effect of inter-row ground cover treatments on the number of cluster/ shoot in the R-block.

The yield of grapevines was increased by the treatments tested ($P < 0.001$). The yield of plastic sleeves and ryegrass treatments were respectively, 140% and 88% higher than that in control. Again, there were no differences between yield in Barrier nil, Barrier U2, fescue and control treatments (Figure 5.11).

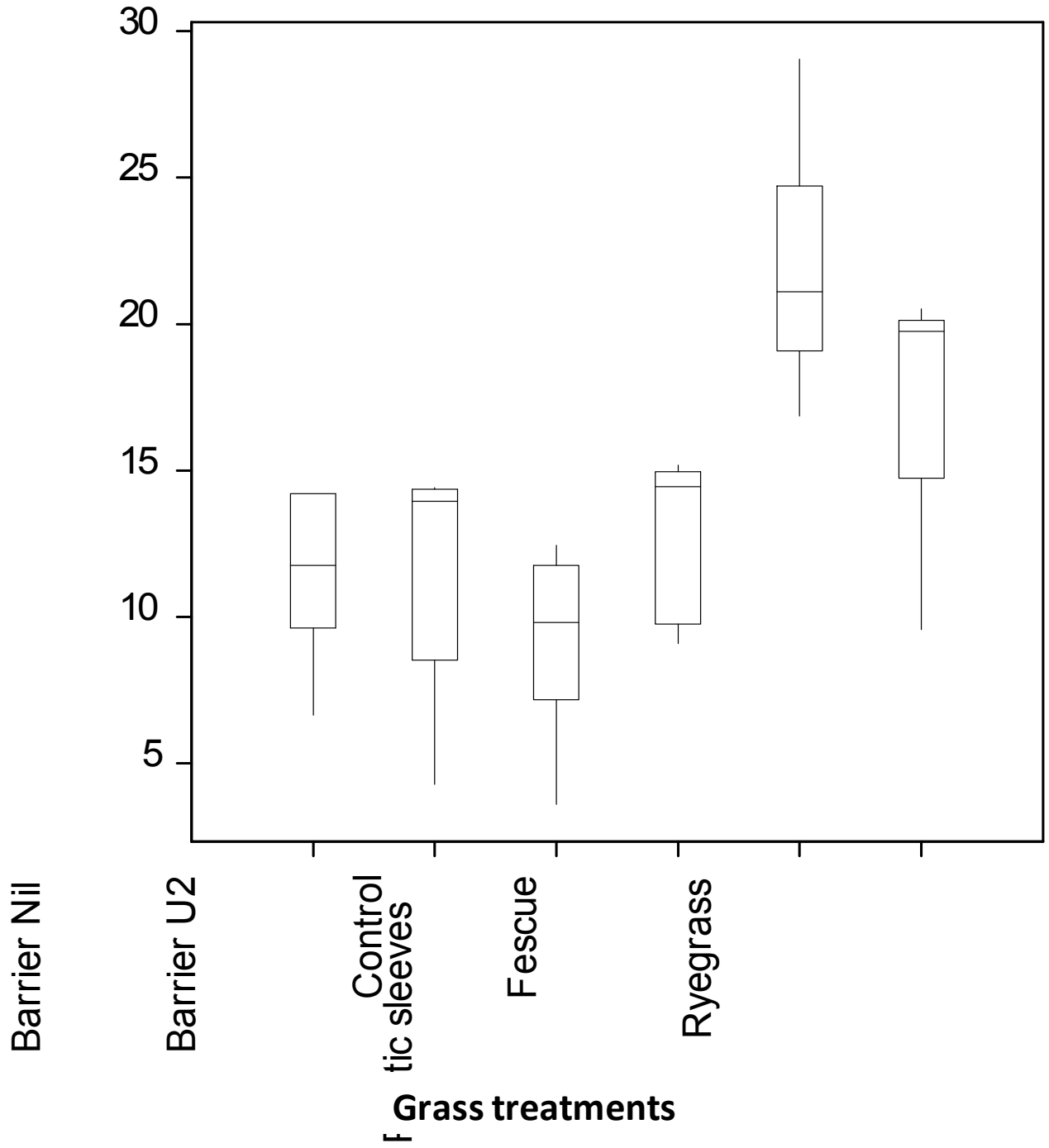

Figure 5.11 Effect of inter-row ground cover treatments on grapevine yield in the R-block.

5.4.3.2 Effect of inter-row ground cover treatments on wētā density

The overall mean initial wētā density was 2.4 individuals/m^2. There were no significant differences between treatments for their initial wētā density. However, final wētā densities were significantly lower in Barrier U2, Matrix SE and control treatments compared to those with plastic sleeves (Table 5.6). There were also significant changes in densities between treatments in the R- block.

Table 5.6 Effect of inter-row ground cover on wētā densities at different sites and their combined means

Treatment/ location	Initial wētā density/m^2			Final wētā density/m^2			[1]Log_{10} Final/Initial density		
	R block	N block	Combined mean	R block	N block	Combined mean	R block	N block	Combined mean
Control	2.58	1.39	1.99	1.33	1.50	1.42	-0.28 (0.52)	0.00 (1.00)	-0.34 (0.46)
Barrier U2	2.83	1.72	2.28	1.22	2.0	1.61	-0.39 (0.41)	-0.01 (0.98)	-0.20 (0.63)
Easton MaxP	2.33	1.58	1.96	1.53	1.22	1.38	-0.14 (0.72)	-0.13 (0.74)	-0.13 (0.74)
Plastic sleeve	1.92	1.94	1.93	2.06	1.78	1.92	0.05 (1.12)	-0.05 (0.89)	0.00 (1.00)
Matrix SE	2.44	1.72	2.08	1.08	1.22	1.15	-0.36 (0.44)	-0.13 (0.74)	-0.24 (0.58)
Mean	2.42	1.67	2.05	1.44	1.54	1.49	-0.22	-0.06	-0.14
[2]LSD (5%)	1.14	1.30	0.95	0.54	1.16	0.86	0.26	0.38	0.39
P - value	0.542	0.921	0.839	0.014	0.549	0.309	0.017	0.906	0.563
P – values for the effect of experimental sites									
			0.026			0.638			0.149

[2]LSD = Least significant difference at 5% probability threshold; [1]Log_{10} Final/Initial wētā density = change in wētā density

5.5 Discussion

5.5.1 Effect of endophyte infection on grasses grazed by wētā

The mutualistic association between fungal endophytes and grasses protects the latter from most insect herbivores (Leuchtmann, Schmidt, & Bush, 2000; Pennell & Ball, 1999). The effect of this association on insects from the order Orthoptera has been poorly studied, although this order contains many of the economically important grassland pests (Barker et al., 2015b; Branson et al., 2006). In this study, extensive feeding damage was found on the endophyte-free grasses (Barrier Nil and Ruanui) but very limited damage was recorded on Barrier U2 and fine fescue, both of which contained endophyte. The *Epichloë* infection in the latter prevented continued feeding by the wētā in both choice and no-choice experiments. Similar reports of reduced feeding damage sustained by an endophyte-infected grass presented to a large orthopteran, *Locusta migratoria* (Linnaeus, 1758), was reported by Lewis et al. (1993). However, subsequent studies using grasshoppers reported positive, neutral or negative effects of *Epichloë* infection on herbivory (Afkhami & Rudgers, 2009; Crawford, Land, & Rudgers, 2010; Saikkonen, Helander, Faeth, Schulthess, & Wilson, 1999; Zhang et al., 2012). The Barrier U2 used in this study had been developed through rigorous selection for high concentrations of *E. uncinata* U2 strain and this probably contributed to the reduced damage sustained by grasses with which it was associated (Barker et al., 2015a; Barker et al., 2015b).

In the current work, deterrence was induced in the endophyte-infected grasses after the first few bites by the insect, and as this feeding occurred at the bases of the stems, they fell to the soil surface. Thus, continued feeding attempts on other endophyte-infected plants in the same no-choice experiment resulted in large number of stems being severed. Losses due to insect herbivory in endophyte-infected grass hosts usually occur because the endophyte which the plant contains does not affect the herbivore. Alternatively, the toxin present does have the potential to impact the herbivore but its concentration is too low to be effective (Ball & Tapper, 1999; Clement et al., 2005; Clement, Hu, Stewart, Wang, & Elberson, 2011; Easton, Lyons, Cooper, & Mace, 2009; Faeth & Saikkonen, 2007; Patchett et al., 2011). Environmental factors such as light, soil nutrient level and moisture can also limit the endophytic production of toxic alkaloids that deter herbivory (Bultman & Conard, 1998; Faeth & Saikkonen, 2007). In this work, these factors were probably not responsible for the plant losses, but they were a consequence of unsuccessful feeding attempts by wētā due to the toxins produced by the endophytes. Barrier U2 contained high concentrations of loline alkaloid derivatives while fine fescue contained lolitreme B and ergovaline.

The dry matter yield of Barrier U2 has been previously reported to be higher than that of Barrier Nil when exposed to the insects, *C. zealandica, H. arator, T. commodus* and *Lepidogryllus* sp., in laboratory and field experiments (Barker et al., 2015a; Barker et al., 2015b; Patchett et al., 2011). Here, dry matter yield of Barrier U2 was higher than that of Barrier Nil only in the choice experiments but there was no difference in the no-choice work. This was because the insect moved away from the endophyte-infected grasses after the first bite onto the endophyte-free ones when there was a choice. In contrast, the absence of alternative food in the no-choice experiments led to high biomass losses resulting from the continued excision of most plants. The rate of re-growth in excised grasses was not rapid enough to compensate for the lost parts. This differs from the results of McNaughton (1979), which showed substantial re-growth in grasses after insect feeding. But Afkhami and Rudgers (2009) later reported that biomass yield of grasses exposed to insect herbivory was dependent on the grass genotype and not the presence of endophytes. Hence, monocultures of this grass could suffer significant yield losses when an outbreak of such chewing orthopterans occurs. The benefits of these grasses can be harnessed in locations with such insects or when their outbreak is anticipated, by planting strips of endophyte-free host to trap them, thereby minimising losses in the endophyte-infected grasses. This findings however, suggests that endophyte-infected grasses may be suitable for deterring (or 'pushing') orthopteran pest out of vineyards or orchards when used as inter-row ground cover.

5.5.2 Loline alkaloid derivatives and wētā

Loline alkaloids possess a broad spectrum of insecticidal activity and usually contribute to endophyte-mediated insect resistance in grasses (Ball & Tapper, 1999). As expected, the increased total loline alkaloid concentration of wētā-wounded Barrier U2 deterred wētā from continued grazing. Derivatives of this group of alkaloids (NAL, NANL, NFL and NML) have been confirmed to have diverse detrimental effects on insects when they feed on *E. uncinata*-infected grasses (Ball & Tapper, 1999; Jensen, Popay, & Tapper, 2009; Patchett et al., 2008). Of these four derivatives, concentrations of NFL and NAL above 2000µg/g and 450µg/g plant dry weight respectively, are necessary for feeding deterrence to occur (Bryant, Cameron, & Edwards, 2010; Patchett et al., 2008; Popay & Thom, 2009; Schardl et al., 2013).

In both choice and no-choice experiments, the concentration of NFL in Barrier U2 in plants not exposed to the insect was below the minimum required to deter herbivory but higher in those with insect wounds. However, NAL concentration was above the minimum needed to deter insects even when the plants were not wounded by wētā. Thus, NFL probably contributed most to the feeding

deterrence observed here and its low concentration in the absence of herbivory accounted for the plant excisions reported in this study.

Reduced survivorship, oviposition and growth have been observed in some insects fed on grasses and artificial diets containing loline alkaloid (Barker et al., 2015b; Clement et al., 2011; Popay & Thom, 2009). Similarly, volatiles emitted by *Hypocrea lixii* F3ST 1-inocluated onions reduced the survival of *Thrips tabaci* Lindeman on the latter compared to endophyte-free controls (Muvea et al., 2015). However, the present study did not establish any such effects for wētā. The ability of this insect to survive for more than 7 days without feeding (J. Nboyine, pers. obs.), especially, when its diet is changed, may have contributed to the lack of remarkable adverse effect of lolines on its growth and survival during these experiments. In the no-choice experiment, introducing the wētā to grasses, after initially maintaining the insects on carrots (*Daucus carota* L.) in the laboratory, affected their initial feeding and this contributed to the observed weight loss. When feeding started on the endophyte-free grasses, wētā were unable to recover the lost weight before the end of the experiment. In contrast, insects used in the choice test did not suffer this initial weight loss because they were maintained on grasses before they were used for the experiment. However, the weight change was minor and not significant. Hence, longer periods of exposure to infected grasses are needed before a determination can be made on the long-term effects of loline alkaloids on this insect.

5.5.3 Implication for deterrence of wētā from vineyards

In the field study, although an equivalent number of buds were laid down in each treatment, it was only vines protected with sleeves that recorded reduced wētā damage to their buds. Grapevines have compound buds (i.e., primary, secondary and tertiary buds). The primary bud begins sprouting at budburst, but when it is damaged by frost or wētā, the less productive secondary buds replaces it. The tertiary bud similarly replaces damaged secondary but produces only tendrils (Creasy & Creasy, 2009; Keller, 2010b). Shoots arising from the secondary are less productive. Here, the sleeves denied wētā access to the young developing primary buds resulting in an increase in the number of clusters/shoot, bunches/vine and grape yield. In contrast, the primary buds on vines in the grass treatments were mostly replaced by secondary ones after wētā damaged the former. This resulted in an about 40% decline in numbers of clusters/shoot and bunches/ vine. Yield was consequently affected in those treatments.

The efficacy of endophytes to confer protection on their grass hosts has resulted in this association being exploited for protecting the host plants (Barker et al., 2015b; Patchett et al., 2008). This work

further examined the possibility of this protection repelling insects from vineyards when they become starved because grasses in the inter-row which serve as alternate food are infected with endophytes. Here, the final wētā density was highest in the sleeve treatment but reduced in the control and grass treatments. The extents of reductions were highest in Barrier U2 (59%) and Matrix SE (56%) treatments. Apart from the latter, this reduction did not correspond to any yield increase. The number of cluster/shoot, bunches and yield of vines in Matrix SE treated plots were not significantly different from those in the sleeve protected vine. Perhaps, the grasses used in this study should have been planted earlier than the September 2015 sowing period. Vine are pruned in Autumn (March – May) and there is not much green vegetation inside vineyards until October (budburst), apart from plants growing in the inter-row. Hence, having the endophyte-infected grasses replacing the inter-row vegetation around that period might have resulted in the insect moving out of the treated area to other places because of food scarcity. This notwithstanding, the observations from Matrix SE treatment hints of the potential of using endophyte-infected grasses to 'push' this insect out of vineyards.

Conclusions

In summary, the bio-pesticidal effects of toxins produced by endophyte-infected grasses on insect pests have been demonstrated in many studies. However, the effect of unsuccessful feeding by large chewing orthopterans on the plant and its biomass after they are deterred has not been examined. This is because these studies were interested in deterrence effects of the endophyte on insects or the feeding behaviour of the insects used did not cause significant plant excisions. However, this study showed that significant yield losses could occur in endophyte-mediated herbivore resistant grasses after the initial bites, although the presence of the toxins deterred further feeding. The losses reported here contrast other similar experiments in which herbivory occurs because of the low quality and quantity of alkaloids or the presence of an endophyte which does not produce anti-herbivory toxins (Clement et al., 2011; Faeth & Saikkonen, 2007; Lopez et al., 1995; Popay & Thom, 2009).

The potential of such grasses replacing inter-row vegetation and repelling orthopteran pest such as wētā has not previously been considered. This work hints that, endophyte-infected grasses could potentially be used to repel wētā from vineyards if they are established ahead of economic damage. However, further experiments are needed to examine the best way of integrating endophytes into orthopteran pest management strategies in vineyards because they can also push the pest onto the vines.

Chapter 6

Discussion & conclusions

About 12% of the world's land area is used for crop production (i.e., >1.5 billion hectares), with larger areas potentially suitable for agriculture being covered by forests, protected for environmental reasons or being part of urban areas (FAO, 2015). Approximately 90% of undeveloped potential agricultural land is located in Latin America and sub-Saharan Africa, while southern and western Asia, and northern Africa have almost none left for agricultural expansion (FAO, 2015). Only 500 million hectares of agricultural land is dedicated to agricultural heritage systems that still maintain their unique traditions with a combination of social, cultural, ecological and economic services that benefit humanity (TEEB, 2015). The remainder relies on chemical inputs. For instance, it is estimated that a mean of over 100 kg of fertilizers (nitrogen, phosphorus, potassium) and about 3 – 12 kg of pesticides are applied annually, per hectare of arable land, in order to sustain and/or increase productivity globally (FAO, 2015). The reliance on these inputs is because vast areas of land worldwide are cropped to a few monocultural species (Bianchi et al., 2006; Rusch et al., 2016). This has resulted in major biodiversity losses in farmland which impacts on important ecosystem functions including natural pest population reduction (Cardinale et al., 2012; Cardinale et al., 2006; Loreau et al., 2001; Mace, Norris, & Fitter, 2012). Hence, many insects are elevated to pest status in these cropping systems. For instance, a New Zealand endemic insect, a wētā, was recently elevated to the status of a pest in vineyards in the Awatere Valley, Marlborough, after a rapid change in land use from native vegetation to pastures and in the last three decades, to vines (Joanne Brady, pers. Comm. 2014, Constellation Brands NZ; http://www.marlborough.govt.nz/Environment; State of the Environment, 2008).

Wētā are generally insects of conservation interest because all the species belonging to this assemblage are endemic to New Zealand and because there are declines in the populations of some species (Sherley, 1998; Sherley et al., 2010). For this reason, periodic reviews of their conservation status are undertaken, based on the availability of new data on their distribution (Trewick et al., 2016; Trewick et al., 2012). Mitigating wētā damage in affected vineyards in the Awatere Valley will therefore require adopting a management approach that will not worsen the conservation status of the species damaging vines. This thesis combined a series of laboratory and field work to develop non-pesticide alternatives for reducing wētā damage to vines, with practical implications for other orthopteran soil-dwelling insect pest in perennial cropping systems.

6.1 Study approach and outcomes

The first experimental chapter (Chapter 2) comprised two major parts. The first established the number of wētā species associated with vine damage and proceeded to identify the exact species, thus enabling its conservation status to be determined. This involved phylogenetically analysing COI sequences obtained from wētā specimens collected from vineyards and using morphological keys to determine the exact species associated with vine damage. A limitation of the phylogenetic analysis was the limited number of quality sequences that could be used for the analysis (i.e., 12 out of 34 specimens analysed). This was because of the poor quality of DNA obtained from most specimens. Similar difficulties in obtaining quality DNA from other wētā species and orthopterans have been reported (Leung, Cruickshank, & Hale, 2012) M. McDonald, pers. comm. January 2015). However, combining DNA barcoding and morphological keys made it possible to accurately identify the species causing damage in the Awatere Valley as *H.* sp. 'promontorius'. This species is not threatened but has a restricted habitat range (Trewick et al., 2016). The second part of Chapter 2 provided basic data necessary for developing strategies for mitigating damage by this wētā. It showed that higher densities of this insect were present in vineyards than in other non-vine habitats. This was thought to be because of the year-round availability of food and the presence of adequate moisture needed for eggs to develop and hatch during the breeding season. In vineyards, densities were higher under-vines than in the inter-rows, but this did not change between the edge and centre. These findings highlighted the need to adopt conservation management for this wētā. It also suggests that management actions must be targeted at the under-vine area to be effective. However, the ideal strategies, if widely adopted, must not significantly kill the high numbers of wētā in vineyards because that could potentially result in their becoming classified as 'threatened species'. This is because *H.* sp. 'promontorius' is restricted to only few locations in the Marlborough region (Townsend et al., 2008; Trewick et al., 2016) and the arid conditions in habitats other than vineyards, especially in the dry summer and autumn months, do not support the survival of this insect.

Chapter 3 established the range of plant species present in the diet of this wētā. The results showed that wētā fed on plants from more than 30 families and 44 genera. An analysis of the plants present in the diet relative to those abundant in vineyards showed that this insect's choice of plant food was probably influenced by nutrient requirements. *H.* sp. 'promontorius' is an omnivore with preference for protein food, but the use of pesticides for pest control (e.g., leafrollers etc.) in the vineyards probably limited the availability of arthropods that could be used as sources of animal food. It therefore relied on vines and other plant species present in vineyards to supplement the proteins and other nutrients derived from the grasses. Diet mixing is a common feeding behaviour among such generalist feeders to optimise the nutrients gained and to minimise the effect of toxic plant defences on them (Ali & Agrawal, 2012; Bernays et al., 1994). Interestingly, vines were detected in

the diet of wētā in all seasons, contrary to initial thoughts that this insect fed on vines only at budburst. The findings suggest that establishing trap plants rich in protein could potentially reduce feeding damage to vines at budburst.

Chapter 4 focused on identifying appropriate habitat manipulation strategies that could be used to reduce damage. Based on knowledge about wētā distribution in vineyards and the dietary requirement of this insect, three under-vine (pea straw mulch, mussel shells, tick beans) and two inter-row (existing vegetation, tick beans) treatments were tested for their efficacy to mitigate damage. At budburst, wētā mostly fed on under-vine beans instead of vine buds. This significantly increased yield in that treatment. However, the presence of inter-row beans did not result in significant reduction in bud damage. This contrasting effect was probably due to the distribution of the insect in vineyards. With higher wētā numbers under vines than in the inter-rows, and the closer proximity of vines to their under-vine burrows than in the inter-row beans, vine buds were most the damaged compared to the inter-row beans. Spreading mussel shells under vines reduced bud damage by serving as a physical barrier against burrow exit by wētā at night. This treatment was highly effective because most wētā were located under vines. They subsequently abandoned their burrows in the shell treatments after making alternative exit routes, resulting in reduced wētā numbers at the end of that experiment (J. Nboyine, pers. obs.). Shells also conserved moisture and suppressed weed growth. In contrast, the straw mulch was not effective at preventing damage. However, it also suppressed weed growth and conserved moisture for the vines. In general, yields from the under-vine bean, shell and plastic sleeve treatments did not differ significantly. Thus, winegrowers have the option either using beans or shells to manage wētā damage or complement the current sleeve management strategy with either strategies. Adopting under-vine beans and shell treatments have the added advantage of increasing the assemblage of natural enemies of pest species in vineyards (i.e., beans) (Nuessly, Hentz, Beiriger, & Scully, 2004) or conserving moisture and suppressing weed growth (i.e., shells) (Guerra & Steenwerth, 2012; Steinmaus et al., 2008). Besides, the sleeves are non-degradable, thus polluting the environment when they detach from vines. They also need annual repairs and/or replacements.

The last experimental chapter (chapter 5) studied the potential of using endophyte-infected grasses to repel wētā from vineyards. This initially involved laboratory experiments to test feeding deterrence against wētā by the grasses, followed by a replicated experiment in two separate vine blocks. The endophyte in the grasses tested had been proven in laboratory and field work to be effective at deterring feeding by a range of insect pests in pastures (Barker et al., 2015a; Barker et al., 2015b; Patchett et al., 2008; Patchett et al., 2011). However, this work showed that although the endophyte-infected grasses deterred feeding, they still sustained significant biomass losses when they were presented to wētā in a no-choice experiment. This was because the grasses were fell over

after the first wētā bite, which mostly occurred at the base of the stems. In contrast, paired choice test with endophyte-free and endophyte-infected grasses found the latter not sustaining significant damage. The concentration of loline alkaloids (i.e., alkaloids responsible for deterrence in this case) increased in injured grasses compared to non-injured ones. When similar endophyte-infected grasses were tested for their wētā repellency effect in vineyards, the results were inconclusive. This was because planting of the grass treatments was delayed and establishments in one of the vine blocks was poor at the time of budburst. This notwithstanding, there were reductions in wētā numbers in all endophyte-infected grass treatments at the site with good grass establishment. This reduction in density corresponded to a yield increment in only one endophyte treatment, i.e., Matrix SE. Overall, the results from the field experiments suggested that if the grasses were established earlier, wētā would have been repelled from the treated areas.

6.2 Implications for wētā management

Before this PhD work commenced, *H.* sp. 'promontorius' was assigned a 'Naturally Uncommon' threat status based on the New Zealand threat classification system (Trewick et al., 2012). According to the latter, Naturally Uncommon refers to 'taxa whose distribution is naturally confined to specific substrates, habitats or geographical areas, or taxa that occur within naturally small and widely scattered populations' (Townsend et al., 2008). Such taxa may have a stable or increasing population, or they may have more than 20,000 mature individuals occupying an area less than 100,000 ha (Townsend et al., 2008). For *H.* sp. 'promontorius', its population was known to be restricted to areas between Marfells Beach and Cape Campbell as well as a few other nearby places in the Marlborough region (Johns, 2001; Trewick et al., 2012). However, a recent revision of the threat status of orthopterans in New Zealand, based on 2014, data placed this wētā in a 'Not threatened' category, but its habitat range was still maintained as restricted (Trewick et al., 2016). To protect the population in vineyard and prevent this wētā from becoming threatened, winegrowers opted to use plastic sleeves, instead of pesticides, to protect vines from this insect's damage.

Wētā are pest because of the transformation of their habitats to vineyards. Applying principles of community ecology that are relevant to developing an ecologically-based integrated pest management strategy (Brown, 1999; Ekström & Ekbom, 2011) is therefore suggested as key to sustainably mitigating wētā damage in vineyards. Habitat manipulation approaches such as diversification of vineyards to include plants from more than two families should be adopted. This is because the dominance of vineyards by plants from two families – Vitaceae and Poaceae – is not heterogeneous enough to prevent damage to vines by an omnivore such as wētā. Increasing plant diversity in an agricultural landscape protects the host (e.g., vines) by masking it. Insect orientation towards host plants is affected in very diverse landscapes because of the visual attributes of plants present, such as colour (Randlkofer, Obermaier, Hilker, & Meiners, 2010). Also, damage levels in the

main crop reduce with increases in the fraction of non-crop vegetation in the environment (Potting, Perry, & Powell, 2005). Fortunately, vineyards, like orchards, offer ideal environments for building and maintaining such stable and diverse plant communities without decreasing the area dedicated to the main crop (Brown, 1999). As a starting point, the composition of the present inter-row vegetation could be extended to include species from the families Caryophyllaceae, Urticaceae, Aseraceae, Brassicaceae etc. (see Table 3.2 in Chapter 3 for a full list of potential families). The under vines (where most wētā live) could also be sparsely planted with species from some of those families, especially those that flower in spring. This will ensure that, in addition to wētā control, the flowers of species from those families can contribute towards enhancing natural enemy abundance for control of other vine pest such as leafrollers, leafhoppers, thrips etc. (Altieri et al., 2005; Begum, Gurr, Wratten, Hedberg, & Nicol, 2006; Berndt & Wratten, 2005; Berndt et al., 2002; Berndt et al., 2006; Landis et al., 2000). To reduce cost and ensure sustainability, species that can persist in vineyards for more than a year should be selected.

Alternatively, a 'haven' or 'wētā bank' or 'wētā refuge' could be created for this insect outside vineyards as a long-term strategy. This could involve demarcating a 4.8 m wide area close to at least two of the four edges of a vine block and creating the conditions identified in Chapter 2 as conducive for the survival of wētā. The wētā refuge can be planted with two rows of vines or shrubs/trees (e.g. *Tilia* spp., *Prunus* spp., *Quercus* spp. etc). These are important, not just as a source of food for wētā, but also because this insect attract mates for mating on trees during the breeding season which occurs in January/February (Gwynne, 2004). Hence, to ensure continuous reproduction in the wētā refuge, trees/shrubs must be included in the range of plants sown. Fruit trees may also be considered and planted to serve the dual purpose of being substrate for wētā reproduction and fruits for human consumption. The ground cover in this refuge could be a mixture of some of the species mentioned in Table 3.2 as well as tick beans. However, relatively large bare areas under the trees/shrubs/vines must be maintained for this wētā to make burrows. To ensure egg hatch as well as the continued availability of food, the wētā refuge should be irrigated whenever necessary, particularly in the dry summer months and in autumn. Wētā could then be translocated, at least in the first year, from the vineyards into this area. Ideally, moving the many nymphs present in October – February, and many adults as well, will ensure a rapid population build-up. The adults will mate within this period and lay eggs which will subsequently hatch in September, while the immature ones will mature in the next breeding season, reproducing then. Using the borders of vineyards to establish such a 'wētā bank' has the added advantage of serving as a non-crop habitat for natural enemies of many vine pest (Altieri et al., 2005; Gurr et al., 2003; Shelton & Badenes-Perez, 2006). These contribute to pest population suppression, especially at the edges of vineyards near the refuge.

At the community level, efforts at making the patches of a few selected non-agricultural areas in the Awatere Valley suitable for wētā and other endemic species could be an extension to the proposed 'wētā refuge' or 'haven' concept. This will require the concerted efforts of winegrowers, local authority/council and the Department of Conservation. The arid nature of non-agricultural habitats that exposes some of them, mostly grasslands, to summer fires could be improved by occasionally irrigating such places. This will guarantee the year-round availability of plant food for wētā and other fauna. It will also preserve some of the native fauna and flora present in those areas, thus protecting indigenous biodiversity. Although this may be expensive, the consequences of biodiversity losses are greater (Rockström et al., 2015; Rockström, Steffen, Noone, Persson, Chapin III, et al., 2009; Steffen et al., 2015; TEEB, 2015). For instance, declines in numbers of pollinators due to the loss of their habits is resulting in loss of wild plant species that rely on them for pollination, with consequences on ecosystem stability (Biesmeijer et al., 2006; Potts et al., 2010). Already, a number of schools, landowners, communities and government agencies across New Zealand are involved in over 3,500 projects aimed at rejuvenating indigenous ecological ecosystems (full information: www.naturespace.org.nz). Cues could be taken from these projects to commence one for protecting not just wētā in the Awatere Valley, but other indigenous invertebrate species.

After a successful establishment of wētā refuges, wētā in vineyards can be 'pushed' out by replacing the inter-row vegetation with endophyte-infected grasses. As was found in Chapter 5, this strategy is also a long-term one because the grasses must be fully established in the vineyards to be effective. This approach to pushing wētā out of vineyards is harmless because the alkaloids responsible for deterrence do not kill the insect after the initial bites. Pushing wētā out of vineyards can also be facilitated by spreading mussel shells under vines. Apart from reducing numbers under vines, the shells will suppress the growth of weeds that could have served as alternative food amidst an endophyte-infected grass inter-row ground cover; thus facilitating the rate at which wētā will move out of the vineyard.

6.3 Conclusions

The principle of this work was to contribute to reducing further irreversible damage to our biosphere and thus, preserve the natural resource base on which future food security depends (Rockström et al., 2015; Rockström, Steffen, Noone, Persson, Chapin, et al., 2009; Rockström, Steffen, Noone, Persson, Chapin III, et al., 2009; Steffen et al., 2015; TEEB, 2015). This informed the overall aim of this PhD programme, which was to reduce pesticide use in vineyards by developing ecologically-based integrated pest management strategies for a New Zealand endemic insect pest, a wētā. Although this work concerned beverage production, the management techniques developed here are appropriate for perennial crops such as tree or bush fruits. This thesis showed that the wētā damaging vines, *H.*

sp. 'promontorius', was not a threatened species. However, its density was about 100 times higher in vineyards than in non-vine habitats. Being a taonga species, there is the need to closely monitor the populations in vineyards and other agricultural lands to prevent it from unknowingly slipping i nto a threatened species status. This is because pesticides that can harm them are used in some of these agricultural habitats, although not to manage wētā. Within vineyards, higher numbers of this insect were found under vines than in the inter-rows, but there were no differences between densities at the edges and centres of vineyards. This was because under-vine areas were mostly bare, with high soil moisture and low compaction.

The plants in wētā diet comprised species from 30 families and 44 genera. Although grasses and vines were dominant in this landscape, plants from other families were important in the diet of this insect. Diet mixing is a feeding strategy common to omnivorous and generalist insect feeders that is aimed at deriving optimum nutrients from their food and also to protect the herbivore to some extent from toxic plant defence chemicals (Bernays et al., 1994; HaÈgele & Rowell-Rahier, 1999). Protecting vines from wētā and other generalist insect pests therefore requires shifting away from the current inter-row ground cover of plants from a single family to a mixture of species from different families.

This thesis also concluded that habitat manipulation strategies such as provision of alternative food (tick beans) for pests and mulching under vines with mussel shells can be very effective in reducing wētā damage. For species planted as alternative food, the under-vine location was found to provides maximum effect. Apart from protecting vines from wētā damage, the tick beans used in this work can potentially attract over 27 species of natural enemies of insect pests, thus potentially reducing populations of other pests (e.g., thrips, leafhoppers, leafrollers) in vineyards (Nuessly et al., 2004; Stoddard, Nicholas, Rubiales, Thomas, & Villegas-Fernández, 2010). Shells also suppresses weed growth and conserve water in vineyards, thus reducing cost associated with irrigating vines during periods of drought, especially in summer (Guerra & Steenwerth, 2012; Jacometti et al., 2007a, 2007b).

This work further demonstrated the potential of repelling wētā from vineyards with endophyte-infected grass inter-row ground cover. Deterrence was proven in laboratory feeding experiments, but time constraints did not allow for this to be fully demonstrated in field trials. However, a single year's data from one of the sites for the field work showed that this concept is feasible.

Overall, the work in this thesis suggests that sustainable non-pesticide based approaches to wētā management are possible. They can be used alone or a number of them can be combined to achieve the desired outcome.

6.4 Future work

This PhD work was constrained by the three-year time limit and funding. Hence, all ideas could not be investigated. In terms of biology and habitat distribution, future work could focus on providing a taxonomic description for this wētā. A survey could be undertaken around Marlborough to quantify the total areas (i.e., agricultural and non-agricultural) that are inhabited by this wētā. Such information, though the first of its kind for this species and many other wētā, could guide an informed decision on the level of threat to this taonga species. Species of wētā found in less than 10,000 ha of non-agricultural areas are considered as being threatened (Taylor-Smith et al., 2016; Townsend et al., 2008). Hence, if higher proportion of the area inhabited by this insect is subjected to agricultural activities, with inhabited non-agricultural areas being less than 10, 000 ha, then action may be needed to protect them. This is particularly important if high volumes of agricultural pesticides are used in farms occupied by wētā.

Here, the reasons for wētā becoming a pest in vineyards were identified and a number of strategies tested for their efficacy to reduce damage. However, the efficacy of endophyte-infected grasses to repel wētā from vineyards was tested in only one season. Although this was done at two sites, the poor establishment of the grasses affected the outcome of the results for one site. This aspect could therefore be validated further, by repeating the experiment and collecting data over at least three seasons and at more sites.

Finally, combining the results from all the experimental chapters to design a kind of 'push – pull' system for wētā management can be considered in the future. Of course, this will take more than a year for the 'wētā refuge' idea which is intended to serve as the 'pull' factor to attract most wētā out of vineyards and also, for the endophyte-infected grasses to establish and produce the desired 'push' effect. The efficacy of the 'push' factor can be enhanced by spreading mussel shells under the vines. Although designing such a system appears time consuming, the desired outcome of reducing plastic waste (i.e., from sleeves that detach from vine stems) and the benefits to the environment and mankind, makes it worth pursuing.

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