

When host-plant resistance to a pest leads to higher plant damage

J. A. Nboyine¹ · D. Saville² · S. Boyer^{1,3} · R. H. Cruickshank⁴ · S. D. Wratten¹

Received: 25 April 2016 / Revised: 8 June 2016 / Accepted: 15 June 2016 / Published online: 24 June 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract The effects of the association between grasses and fungal endophytes on orthopterans are very poorly studied although they are important grassland pests. Here, the endemic New Zealand weta, *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 effects of this interaction on the grass. The insect was 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*. In 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 ($P < 0.05$). The current work shows that endophyte-infected grasses can sustain high plant losses when attacked by an orthopteran insect in the absence of an alternative food source. This contrasts other endophyte/herbivory experiments in which high herbivory occurs because chemical plant defences are at a low concentration or the endophytes have other non-toxin roles in the plant.

Keywords *Epichloë uncinata* · Loline alkaloids · *Festulolium loliaceum* · Biomass loss · Orthoptera · Pest management

Key message

- The effect of the symbiotic association between endophytic fungi and grasses on insects in the order Orthoptera is poorly studied, although the latter contain many economically important grassland pests.
- This work shows that endophyte-infected grasses can deter feeding by orthopteran insect, but the consequence of unsuccessful feeding by this insect still results in significant yield losses.
- Such losses can be minimized by planting strips of endophyte-free grasses to serve as trap crops when monocultures of endophyte-infected grasses are grown.

Communicated by E. Quesada-Moraga.

✉ J. A. Nboyine
Jerry.Nboyine@lincolnuni.ac.nz

¹ Bio-Protection Research Centre, Lincoln University,
P. O. Box 85084, Christchurch 7647, New Zealand

² Saville Statistical Consulting Limited,
P. O. Box 69192, Lincoln 7640, New Zealand

³ Environmental and Animal Sciences, Unitec Institute of
Technology, 139 Carrington Road, Mt Albert,
Auckland 1025, New Zealand

⁴ Department of Ecology, Faculty of Agriculture and Life
Sciences, Lincoln University,
P. O. Box 85084, Christchurch 7647, New Zealand

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 and Garnett 2014). Apart from killing the target insect pests, insecticides also generate external costs such as killing pollinators and pests' natural enemies (Fernandes et al. 2010; Jones et al. 2014) as well as those associated with the applications themselves, such as the agrochemical, 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 (Shennan 2008; Poppy et al. 2014).

These sustainable practices include the exploitation and enhancement of a plant's ability to defend itself from insect pest attack (Kumari et al. 2006; Ronald 2011; Mortensen 2013). For instance, some plants produce constitutive (i.e. always present) specialized bioactive compounds (alkaloids, cyanogenic glucosides, glucosinolates, phenolics, terpenoids, etc.) that defend them against insects (War et al. 2012; Fürstenberg-Hägg et al. 2013). 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) (Kogan 1994; War et al. 2012; Fürstenberg-Hägg et al. 2013). Certain morphological features (e.g. trichomes, epicuticular waxes) are also constitutive and may be involved in antixenosis (Kogan 1994; Fürstenberg-Hägg et al. 2013). 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 are induced in response to insect feeding or even the release of insect pheromones (Helms et al. 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 et al. 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 and Hume 2011). These toxins are constitutive, but induction also occurs in response to herbivory (Patchett et al. 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 et al. 2011; Popay and Hume 2011). These grasses are infected with strains of

endophyte species from the genus *Epichloë* (Faeth and Saikkonen 2007; Schardi et al. 2013; Guerre 2015). But 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 and 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 (Ball and Tapper 1999; Clement et al. 2005; Faeth and Saikkonen 2007; Afkhami and Rudgers 2009).

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)) (Patchett et al. 2011; Barker 2011; Barker et al. 2014, 2015). 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 (Lewis et al. 1993; Lopez et al. 1995; Afkhami and Rudgers 2009; Zhang et al. 2011). Meanwhile, the occasional outbreaks of these insects result in significant yield losses in the absence of appropriate plant protection measures (Branson et al. 2006).

Here, the endemic New Zealand ground weta, *Hemiandrus* sp. 'promontorius' (Johns 2001) (Orthoptera: Anostostomatidae), and *E. uncinata*-infected *F. loliaceum*, were used to study the effects of endophyte-mediated resistance in grasses on large orthopterans and the effects of this interaction on the biomass yield of the grass. This insect is an occasional pest and possesses strong chewing mandibles that it uses to cause extensive feeding damage to plants (Johns 2001). This work was conducted because preliminary experiments suggested counter-intuitive results in terms of higher levels of damage being sustained by the endophyte-infected grasses than by the controls.

Materials and methods

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 1.

Seeds of these grasses were planted in 300 ml plastic pots filled with sandy loam soil in a glasshouse at Lincoln University, Christchurch, New Zealand. Three weeks after germination, the grasses were thinned to plant stands of 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. 2014, 2015).

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

Life cycle of *H. sp.* ‘promontorius’ and host plants

The life cycle of weta in the genus *Hemiandrus* Ander 1938 is completed in a year but they live for 2 years (Johns 2001). Their developmental cycle is, of course, linked to seasons. Females of *H. sp.* ‘promontorius’ lay eggs in early March (i.e. autumn), and these hatch in late September (i.e. spring). The males and females pass through 9 and 8 nymphal stages, respectively, to become adults at the end of the first year. Each season in the year is mostly dominated by two nymphal stages, but other stages may be present. For the first year in the life cycle of this insect, spring (September–November) has higher proportion of instar 8 for females or 9 for males (from previous year) and instar 1, while summer (December–February) has instars 2–3. Instars 4–5 and 6–7 are present in autumn (March–May) and winter (June–August), respectively (Van Wyngaarden 1995; Nboyine et al. 2016). Females spend

the second year laying eggs, brooding them and taking care of the first instar nymphs, while males fertilize females during this period (Gwynne 2004). In this laboratory study, fourth and fifth instar nymphs collected from the field were used. They were randomly distributed across treatments. The instar stages of insects used were distinguished based on the extent of sclerotization of their terminalia and length of their hind femora (Van Wyngaarden 1995). The insects did not moult during the experiment.

Weta are generally polyphagous. They feed on a wide range of plant species such as the leaves and young tender shoots of grasses (e.g. *Poa annua* L., *Lolium perenne* L.), vines (*Vitis vinifera* L.) and apricots (*Prunus armeniaca* L.) (Wahid 1978; Van Wyngaarden 1995; Nboyine et al. Unpublished data).

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 1. Weta 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 weta 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 was levelled to cover gaps. A single pre-weighed unsexed mid-instar weta 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.

Table 1 Key characteristics of grasses used

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

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 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. One pre-weighed unsexed weta 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 $8 = 2 \times 2 \times 2$ treatment pairs, each in one of eight arenas that were randomized in each block.

Data collection

Weight change of weta

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

Weta survival

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

Damage scores

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

Severed plants

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

Plant biomass

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

Analysis of plants for loline alkaloids

Samples of each grass treatment (each >500 mg fresh weight excluding the roots) were washed and dried with paper towels. They were freeze-dried in liquid nitrogen and ground into fine powder. The method of Yates et al. (1990) as modified by Patchett et al. (2011) was then used to analyse the loline alkaloid content of each sample. Each ground sample of weight 500 mg was shaken vigorously with 10 ml dichloromethane, methanol, ammonia (75:25:0.5) solvent, and 6 mg phenylmorpholine/100 ml of solvent as the internal standard, for 22–24 h. They were then centrifuged at 2000g for 15 min, after which 1 ml of the supernatants was passed through a microfilter (0.45 mm) and held in a 2-ml glass vial for analysis within 24 h by capillary gas chromatography.

Data analysis

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

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

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 et al. 2000) and is designed to compare the relative biomasses of E+ and E− in the presence of weta, after adjustment for their relative biomasses in the absence of weta. 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 weta 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 $\sqrt{2}$). 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 two-sided t test was used to test the null hypothesis H_0 : mean concentration of loline alkaloids in Barrier U2 with weta feeding damage did not differ significantly from those in Barrier U2 without weta feeding damage.

Results

No-choice experiment

There was a significant difference in the rate of feeding damage sustained by the different grasses ($P < 0.001$; Table 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 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 2). When the dry biomass was compared between weta and non-weta infested plants for each grass treatment, there were no significant differences among the four grasses ($P = 0.703$) (Table 2). Weta lost weight by an average of 4–8 % (Table 2). The ratios of final to initial weta weight, and survivorship of weta exposed to the different grass treatments were not significantly different among the treatments.

Ruanui, Barrier Nil and fine fescue did not contain loline alkaloids. However, the fine fescue contained lolitrem 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*-methyl 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

Table 2 For the no-choice experiment, effect of the feeding activities of weta on the damage score and number of severed stems of the grass treatments tested, and weight change and survivorship of weta exposed to the different treatments

Grasses	Feeding damage score	Number of severed stems	Log ₁₀ ratio of plant dry biomass [weta/(no weta)]	Log ₁₀ ratio of weta weights (final/initial)	Weta 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 <i>P</i> 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 <i>P</i> value	<0.001	<0.001	0.626	0.979	

For biomass, the ratio between grass treatments in the presence and absence of weta is presented

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

concentration increased in grasses exposed to weta although this was also not significantly different from that of those not exposed to the insect (Table 3).

Choice experiment

Table 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. Weta 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 4 are both greater than the LSE (5 %) of 2.0). Ruanui was tested in these bioassays and was also significantly more damaged than either Barrier U2 or fine fescue. Examination of the 2×2 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 4). There was also no significant interaction, with the difference between the preference of weta 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 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 weta feeding (compared to no weta 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 weta 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 $\log_{10}(\text{BACI})$ 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 weta (after adjustment for the ratio of remaining dry biomass in the absence of weta).

In this experiment, there was no mortality of the weta, and the weta increased in weight by an average of about 7 % (Table 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 4), nor were any of the main effect or interaction contrasts significant.

In the choice tests, the concentrations of NAL, NANL and NFL were significantly higher in Barrier U2 exposed to weta than Barrier U2 not exposed to weta. There was no significant difference between the concentrations of NML in weta-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 weta was approximately three times higher than in the absence of weta ($P = 0.003$; Table 5).

Discussion

Effect of endophyte infection on grasses grazed by weta

The mutualistic association between fungal endophytes and grasses protects the latter from most insect herbivores (Leuchtman et al. 2000; Pennell and Ball 1999). The effect of this association on insects from the order Orthoptera has been poorly studied, although it contains many of the economically important grassland pests (Branson et al. 2006; Barker et al. 2015). 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 weta in both choice and no-choice experiments. Similar reports of reduced feeding

Table 3 Concentration ($\mu\text{g/g}$) of loline alkaloids in *E. uncinata* U2-infected *F. loliaceum* (Barrier U2) in the presence and absence of weta in a no-choice test ($n = 5$)

Loline alkaloids	Concentration ($\mu\text{g/g}$)		<i>t</i> value (2 tailed)	<i>P</i> value
	Weta present	Weta 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

Table 4 In the choice experiment, effect of weta 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 weta/(no weta)) and weight of the weta (final/initial)

Choice pairs (Endophyte (^a E+) + Non-endophyte (^b E-)-infected grasses)	Mean difference of damage score (E- to E+)	Mean difference of number of severed plants (E+ to E-)	Log ₁₀ ^c BACI of plant dry biomass (E+/E-)	Log ₁₀ Ratio of final weta 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)
<i>P</i> values for 2 × 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
^d LSE (5 %)	2.0	6.6	0.499	0.055

Note that all weta survived in this experiment

^a E+ Endophyte-infected grasses; ^b E- Non-endophyte-infected grasses; ^c BACI (Dry biomass ratio, E+/E-, of grasses presented to weta)/(Dry biomass ratio, E+/E-, of grasses not presented to weta); ^d LSE Least significant effect, for comparing a mean with zero; for the fourth and fifth columns, back-transformed means are given in brackets

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

Loline alkaloids	Concentration (µg/g)		<i>t</i> value (2-tailed)	<i>P</i> value
	Weta present	Weta 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

damage sustained by an endophyte-infected grass presented to a large orthopteran, *Locusta migratoria* (Linnaeus 1758), were reported by Lewis et al. (1993). However, subsequent studies using grasshoppers reported positive, neutral or negative effects of *Epichloë* infection on herbivory (Saikkonen et al. 1999; Afkhami and Rudgers 2009; Crawford et al. 2010; Zhang et al. 2011; Barker 2011). The Barrier U2 used in this study had been developed through rigorous selection for high concentrations of *E. uncinata* U2 strain, and this contributed to the reduced damage sustained by grasses with which it was associated (Barker et al. 2015; Brian J. Patchett, CropMark Seeds Ltd., Christchurch, pers. comm.).

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 and Tapper 1999; Faeth and Saikkonen 2007; Easton et al. 2009; Clement et al. 2005, 2011). Environmental factors such as light, soil nutrient level and moisture can also limit the endophytic production of toxic alkaloids that deter herbivory (Bultman and Conard 1998; Faeth and Saikkonen 2007). In this work, the above factors were not responsible for the plant losses, but they were a consequence of unsuccessful feeding attempts by weta 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 (Patchett et al. 2011; Barker et al. 2014, 2015). 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 minimizing losses in the endophyte-infected grasses.

Loline alkaloid derivatives and weta

Loline alkaloids possess a broad spectrum of insecticidal activity and usually contribute to endophyte-mediated insect resistance in grasses (Ball and Tapper 1999). As expected, the increased total loline alkaloid concentration of weta-wounded Barrier U2 deterred weta 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 and Tapper 1999; Patchett et al. 2008; Jensen et al. 2009). Of these four derivatives, concentrations of NFL and NAL above 2000 and 450 µg/g plant dry weight, respectively, are necessary for feeding deterrence to occur (Popay and Tapper 2006; Patchett et al. 2008; Popay et al. 2009; Bryant et al. 2010; Schardi 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 weta. 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 (Popay et al. 2009; Clement et al. 2011; Barker et al. 2015). Similarly, volatiles emitted by *Hypocrea lixii* F3ST 1-inoculated 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 weta. 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 adverse effect of lolines on its growth and survival during these experiments. In the no-choice experiment, introducing the weta 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, weta 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.

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 (Faeth and Saikkonen 2007; Popay and Thom 2009; Clement et al. 2011; Lopez et al. 1995). Most studies of insect resistance in these grasses have been conducted in Australasia where the diversity of economically important orthopterans in pasture is limited. Hence, the findings of this study have important implications for pest management in locations where occasional severe outbreak of orthopteran pests occurs.

Author contribution statement

JAN and SDW conceived and designed the research. JAN conducted the research. JAN and DS analysed the results and wrote the manuscript. SB and RC improved the experimental design. All authors read and approved the manuscript.

Acknowledgments This study was funded by New Zealand Government's Callaghan Innovation PhD Research and Development Fellowship Grant (Project no. CONB201) in collaboration with Constellation Brands NZ and CropMark Seeds Ltd., Christchurch. The authors gratefully acknowledge the support of Joanne Brady of Constellation Brands NZ. The grass seeds used in this study and the service of testing for loline alkaloids were generously provided by Brian Patchett and Tim Gillanders of CropMark Seeds Ltd.

Funding This study was funded by New Zealand Government's Callaghan Innovation PhD Research and Development Fellowship Grant (Project number CONB 201).

Compliance with ethical standards

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

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

References

- Afkhami M, Rudgers J (2009) Endophyte mediated resistance to herbivores depends on herbivore identity in the wild grass *Festuca subverticillata*. *Environ Entomol* 38:1086–1095
- Azevedo JL, Maccheroni W Jr, Pereira JO, Araujo WL (2000) Endophytic microorganisms: a review on insect control and recent advances on tropical plants. *Electron J Biotechnol* 3(1):15–16
- Ball OJP, Tapper BA (1999) The production of loline alkaloids in artificial and natural grass/endophyte associations. In: Proceedings of New Zealand Plant Protection Conference, New Zealand Plant Protection Society 1998, pp 264–269
- Barker GM (2011) Response of wingless grasshopper to artificial diets and seed containing loline alkaloids. Technical Report 134. Cropmark Seeds Ltd., Christchurch. pp 10. <http://www.grubout.co.nz/documents/>. Accessed 7 Feb 2016
- Barker GM, Patchett BJ, Cameron NE (2014) *Epichloë uncinata* infection and loline content afford *Festulolium* grasses protection from black beetle (*Heteronychus arator*). *NZ J Ag Res* 58(1):35–56
- Barker GM, Patchett BJ, Cameron NE (2015) *Epichloë uncinata* infection and loline content protect *Festulolium* grasses from crickets (Orthoptera: Gryllidae). *J Econ Entomol*. 1–9. doi:10.1093/jee/tou058
- Branson DH, Joern A, Sword GA (2006) Sustainable management of insect herbivores in grassland ecosystems: new perspectives in grasshopper control. *Bioscience* 56(9):743–755
- Brévault T, Heuberger S, Zhang M, Ellers-Kirkb C, Nic X, Massond L, Lib X, Tabashnik BE, Carrière Y (2013) Potential shortfall of pyramided transgenic cotton for insect resistance management. *PNAS* 110(15):5806–5811
- Bryant RH, Cameron NE, Edwards GR (2010) Response of black beetle and red-headed pasture cockchafer larvae to loline alkaloids in meadow fescue roots. *N Z Plant Prot* 63:219–223
- Bultman TL, Conard NJ (1998) Effects of endophytic fungus, nutrient level, and plant damage on performance of fall army worm (Lepidoptera: Noctuidae). *Environ Entomol* 27(3):631–635
- Clement SL, Elberson LR, Bosque-Pérez NA, Schotzko DJ (2005) Detrimental and neutral effects of wild barley-*Neotyphodium* fungal endophyte associations on insect survival. *Entomol Exp Appl* 114:119–125
- Clement SL, Hu J, Steward AV, Wang B, Elberson LR (2011) Detrimental and neutral effects of a wild grass-fungal endophyte symbiont on insect preference and performance. *J Insect Sci* 11:77. Available online: insectscience.org/11.7
- Crawford KM, Land JM, Rudgers JA (2010) Fungal endophytes of native grasses decrease insect herbivore preference and performance. *Oecologia* 164:431–444
- Easton HS, Lyons TB, Cooper BM, Mace WJ (2009) Loline alkaloids for better protection of pastures from insect pests. *Proc N Z Grassland Assoc* 71:151–154
- Faeth SH, Saikkonen K (2007) Variability is the nature of the endophyte-grass interaction. In: Popay AJ, Thom ER (eds) Proceedings of the 6th international symposium on fungal endophytes of grasses. Christchurch, New Zealand, March 25–28, 2006. Grassland research and practice series 13, New Zealand Grassland Association, Dunedin, New Zealand, pp 37–48
- Fernandes FL, Bacci L, Fernandes MS (2010) Impact and selectivity of insecticides to predators and parasitoids. *EntomoBrasilis* 3(1):1–10
- Fletcher LR (1999) 'Non-toxic' endophytes in ryegrass and their effect on livestock health and production. Grassland research and practice series No. 7: 133–139. http://www.grassland.org.nz/publications/nzgrassland_publication_2633.pdf
- Fürstenberg-Hägg F, Zagrobelny M, Bak S (2013) Plant defense against insect herbivores. *Int J Mol Sci* 14:10242–10297. doi:10.3390/ijms140510242
- Godfray HCJ, Garnett T (2014) Food security and sustainable intensification. *Phil Trans R Soc B* 369:20120273. doi:10.1098/rstb.2012.0273
- Guerre P (2015) Ergot alkaloids produced by endophytic fungi of the genus *Epichloë*. *Toxins* 7:773–790. doi:10.3390/toxins7030773
- Gwynne DT (2004) Reproductive behavior of ground weta (Orthoptera: Anostostomatidae): drumming behavior, nuptial feeding, post-copulatory guarding and maternal care. *J Kans Entomol Soc* 77(4):414–428
- Haverkort AJ, Boonekamp PM, Hutten RCB, Jacobsen E, Lotz LAP, Kessel GJT, Visser RGF, van der Vossen EAG (2008) Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. *Potato Res* 51:47–57. doi:10.1007/s11540-008-9089-y
- Helms AM, De Moraes CM, Tooker JF, Mescher MC (2013) Exposure of *Solidago altissima* plants to volatile emissions of an insect antagonist (*Eurosta solidaginis*) deters subsequent herbivory. *PNAS* 110(1):199–204
- Jensen JG, Popay AJ, Tapper BA (2009) Argentine stem weevil adults are affected by meadow fescue endophyte and its loline alkaloids. *N Z Plant Prot* 62:12–18
- Johns PM (2001) Distribution and conservation status of ground weta, *Hemiandrus* species (Orthoptera: Anostostomatidae). Science for conservation 180. New Zealand Department of Conservation, Wellington, New Zealand
- Jones JDG, Witek K, Verweij W, Jupe F, Cooke D, Dorling S, Foster S (2014) Elevating crop disease resistance with cloned genes. *Phil Trans R Soc B* 369:20130087. doi:10.1098/rstb.2013.0087

- Kogan M (1994) Plant resistance in pest management (revised). In: Metcalf RL, Luckmann WH (eds) Introduction to insect pest management. Wiley, New York, pp 76–101
- Kumari DA, Reddy DJ, Sharma HC (2006) Antixenosis mechanism of resistance in pigeonpea to the pod borer, *Helicoverpa armigera*. J Appl Entomol 130(1):10–14. doi:10.1111/j.1439-0418.2005.01024.x
- Leuchtman A, Schmidt D, Bush LP (2000) Different levels of protective alkaloids in grasses with stroma-forming and seed-transmitted *Epichloe/Neotyphodium* endophytes. J Chem Ecol 26(4):1025–1036
- Lewis GC, White JF Jr, Bonnefont J (1993) Evaluation of grasses infected with fungal endophytes against locusts. Ann Appl Biol 14:142–143
- Lopez JE, Faeth SH, Miller M (1995) Effect of endophytic fungi on herbivory by redlegged grasshoppers (Orthoptera: Acrididae) on Arizona fescue. Environ Entomol 24(6):1576–1580
- McDonald TL, Erickson WP, McDonald LL (2000) Analysis of count data from before-after control-impact studies. J Agric Biol Environ Stat 5(3):262–279
- McNaughton SJ (1979) Grazing as an optimization process: grass-ungulate relationships in Serengeti. Am Nat 113(5):691–703
- Mortensen B (2013) Plant resistance against herbivory. Nature Education Knowledge 4(4):5
- Muvea AM, Meyhöfer R, Maniania NK, Poehling HM, Ekesi S, Subramanian S (2015) Behavioral responses of *Thrips tabaci* Lindeman to endophyte-inoculated onion plants. J Pest Sci 88(3):555–562
- Nboyine JA, Boyer S, Saville D, Smith MJ, Wratten SD (2016) Ground weta in vines of the Awatere Valley, Marlborough: biology, density and distribution. N Z J Zool. doi:10.1080/03014223.2016.1193548
- Oerke E-C (2006) Crop losses to pests. J Ag Sci 144:31–43. doi:10.1017/S0021859605005708
- Patchett BJ, Chapman RB, Fletcher LR, Gooneratne SR (2008) Root loline concentration in endophyte infected meadow fescue (*Festuca pratensis*) is increased by grass grub (*Costelytra zealandica*) attack. N Z Plant Prot 61:210–214
- Patchett BJ, Gooneratne SR, Chapman RB, Fletcher LR (2011) Effects of loline-producing endophyte-infected meadow fescue ecotypes on New Zealand grass grub (*Costelytra zealandica*). N Z J Agric Res 54:303–313
- Pennell C, Ball OJ-P (1999) The effects of *Neotyphodium* endophytes in tall fescue on pasture mealybug (*Balanococcus poae*). N Z Plant Prot 52:259–263
- Popay AJ, Tapper BA (2006) Endophyte effects on consumption of seed and germinated seedlings of ryegrass and fescue by grass grub (*Costelytra zealandica*) larvae. In: Proceedings of the 6th international symposium on fungal endophytes of grasses, pp 353–356
- Popay AJ, Hume DE (2011) Endophytes improve ryegrass persistence by controlling insects. Pasture persistence-grassland research and practice series 15:149–156. http://www.grassland.org.nz/publications/nzgrassland_publication_2247.pdf
- Popay AJ, Thom ER (2009) Endophyte effects on major insect pests in Waikato dairy pasture. Proc N Z Grassland Assoc 71:121–126
- Poppy GM, Jepson PC, Pickett JA, Birkett MA (2014) Achieving food and environmental security: new approaches to close the gap. Phil Trans R Soc B 369:20120272. doi:10.1098/rstb.2012.0272
- Ronald P (2011) Plant genetics, sustainable agriculture and global food security: review. Genetics 188:11–20. doi:10.1534/genetics.111.128553
- Saikkonen K, Helander M, Faeth SH, Schultess F, Wilson D (1999) Endophytegrass-herbivore interactions: the case of *Neotyphodium* endophytes in Arizona fescue populations. Oecologia 121:411–420
- Schardi CL, Florea S, Pan J, Nagabhyru P, Bec S, Calie PJ (2013) The *Epichloë* alkaloid diversity and roles in symbiosis with grasses. Curr Opin Plant Biol 16(4) 480–488. doi: <http://doi.org/10.1016/j.jpbi.2013.06.012>
- Shennan C (2008) Biotic interactions, ecological knowledge and agriculture. Phil Trans R Soc B 363:717–739. doi:10.1098/rstb.2007.2180
- Van Wyngaarden F (1995) The ecology of the Tekapo ground weta (*Hemiandrus* new sp., Orthoptera, Anastotomatidae) and recommendations for the conservation of a threatened close relative. Unpublished MSc thesis, University of Canterbury, Christchurch
- Wahid MB (1978) The biology and economic impact of the weta, *Hemiandrus* sp. (Orthoptera: Stenopelmatidae) in an apricot orchard, Horotane Valley. Doctoral dissertation, Lincoln College, University of Canterbury
- War A, Paulraj MG, Ahmad T, Buhroo A, Hussain B, Ignacimuthu S, Sharma HC (2012) Mechanisms of plant defense against insect herbivores. Plant Signal Behav 7(10):1306–1320. doi:10.4161/psb.21663
- Yates SG, Petroski RJ, Powell RG (1990) Analysis of loline alkaloids in endophyte-infected tall fescue by capillary gas chromatography. J Agric Food Chem 38:182–185
- Zhang XX, Li CJ, Nan ZB, Matthew C (2011) *Neotyphodium* endophyte increases *Achnatherum inebrians* (drunken horse grass) resistance to herbivores and seed predators. Weed Res 52:70–78