

Preference of a native beetle for "exoticism", characteristics that contribute to invasive success of *Costelytra zealandica* (Scarabaeidae: Melolonthinae).

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Widespread replacement of native ecosystems by productive land sometimes results in the outbreak of a native species. In New Zealand, the introduction of exotic pastoral plants has resulted in diet alteration of the native coleopteran species, *Costelytra zealandica* (White) (Scarabaeidae) such that this insect has reached the status of pest. In contrast, *C. brunneum* (Broun), a congeneric species, has not developed such a relationship with these 'novel' host plants. This study investigated the feeding preferences and fitness performance of these two closely related scarab beetles to increase fundamental knowledge about the mechanisms responsible for the development of invasive characteristics in native insects. To this end, the feeding preference of third instar larvae of both *Costelytra* species was investigated using an olfactometer device, and the survival and larval growth of the invasive species *C. zealandica* were compared on native and exotic host plants. *Costelytra zealandica*, when sampled from exotic pastures, was unable to fully utilise its ancestral native host and showed higher feeding preference and performance on exotic plants. In contrast, *C. zealandica* sampled from native grasslands did not perform significantly better on either host and showed similar feeding preferences to *C. brunneum*, which exhibited no feeding preference. This study suggests the possibility of strong intraspecific variation in the ability of *C. zealandica* to exploit native or exotic plants, supporting the hypothesis that such ability underpins the existence of distinct host-races in this species.

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14 **Preference of a native beetle for "exoticism", characteristics that**
15 **contribute to invasive success of *Costelytra zealandica* (Scarabaeidae:**
16 **Melolonthinae).**

17 **Abstract**

18 Widespread replacement of native ecosystems by productive land sometimes results in the
19 outbreak of a native species. In New Zealand, the introduction of exotic pastoral plants
20 has resulted in diet alteration of the native coleopteran species, *Costelytra zealandica*
21 (White) (Scarabaeidae) such that this insect has reached the status of pest. In contrast, *C.*
22 *brunneum* (Broun), a congeneric species, has not developed such a relationship with these
23 ‘novel’ host plants. This study investigated the feeding preferences and fitness
24 performance of these two closely related scarab beetles to increase fundamental
25 knowledge about the mechanisms responsible for the development of invasive
26 characteristics in native insects. To this end, the feeding preference of third instar larvae of
27 both *Costelytra* species was investigated using an olfactometer device, and the survival and
28 larval growth of the invasive species *C. zealandica* were compared on native and
29 exotic host plants. *Costelytra zealandica*, when sampled from exotic pastures, was
30 unable to fully utilise its ancestral native host and showed higher feeding preference
31 and performance on exotic plants. In contrast, *C. zealandica* sampled from native
32 grasslands did not perform significantly better on either host and showed similar
33 feeding preferences to *C. brunneum*, which exhibited no feeding preference. This
34 study suggests the possibility of strong intraspecific variation in the ability of *C.*
35 *zealandica* to exploit native or exotic plants, supporting the hypothesis that such
36 ability underpins the existence of distinct host-races in this species.

37 **Key words:** invasive species, native invader, plant-insect interactions, grass grub, feeding
38 preferences, New Zealand

39 **Suggested running head**

40 *Preference of a native beetle for "exoticism"*

41 **Introduction**

42 By widely replacing native ecosystems with more economically productive land, modern
43 intensive agriculture has often been regarded by ecologists as a driver for substantial biodiversity
44 loss (Robinson & Sutherland 2002, Tilman *et al.* 2002, Foley *et al.* 2005). Although detrimental
45 for numerous species, anthropogenic modifications creating novel ecological conditions appear to
46 be beneficial under certain circumstances for some native species. For instance, it is
47 acknowledged that the high diversity of phytophagous insects partially results from evolutionary
48 processes that occur through the action of factors affecting their diet breadth (Gaete-Eastman *et*
49 *al.* 2004), such as the appearance of a new host plant. Hence, the ecological repercussions of
50 anthropogenic-driven modification(s) on native ecosystems are worth investigating to enhance
51 understanding of the insect invasion process. In addition, the comparison of native and invasive
52 congeners is recognised as a useful approach for identifying characteristics that promote
53 invasiveness (Munoz & Ackerman 2011). This approach is perhaps even more useful in this study
54 because the ‘invasive congener’ is native itself and it would not have been subjected to
55 differential environmental and ecological pressures as its congener that are likely to have affected
56 its evolution.

57 In New Zealand, the introduction of exotic pastoral plants has resulted in alteration of the diet of
58 the native coleopteran *Costelytra zealandica* (White) (Scarabaeidae), also known as the New
59 Zealand grass grub or brown beetle. The larvae of this endemic insect feed intensively on the
60 roots of ryegrass (*Lolium* spp.) and white clover (*Trifolium repens*) and as a consequence the
61 species is ranked as a major economic pest in New Zealand (Pottinger 1975, Richards *et al.*
62 1997). Interestingly and in contrast, *C. brunneum* (Broun), a close congeneric species that is
63 rarely found in ryegrass and white clover pastures and remains mostly distributed in native
64 habitats (Given 1966, Lefort *et al.* 2012, 2013). Both *Costelytra* species are considered to be

65 univoltine organisms (Atkinson & Slay 1994) with three larval stages, although it is not
66 uncommon to come across individuals that follow a two-year life cycle in the highest and coldest
67 environments of the southern locations of New Zealand, such as Otago and Southland (Stewart
68 1972, Kain 1975). These two species are sympatric and share similar native hosts, mainly
69 comprising tussock species (Poaceae) commonly found in New Zealand native grasslands (Given
70 1966, Lefort *et al.*, 2012, 2013).

71 The present study aimed to investigate the feeding preferences and fitness response in terms of
72 survival and weight gain of these two coleopteran species, to provide new insights into the
73 mechanisms underpinning the invasion process in *C. zealandica*. The first objective of this study
74 was to perform choice tests where the larvae of both *Costelytra* species were given the choice
75 between a native and an exotic host plant. The second objective was to compare survival and
76 larval growth of two populations of the invasive species *C. zealandica* when exposed to these
77 host plants.

78 **Material and methods**

79 **Insect sampling and plant material**

80 Newly hatched third instar larva, as the most damaging life stage of the invasive species *C.*
81 *zealandica* and the most intensively feeding life stage in *Costelytra* spp. in general, were used for
82 the experiments. No protocol exists to rear *Costelytra* spp. offspring under laboratory conditions
83 and all attempts to do so have been unsuccessful. Therefore, the second best option was to work
84 with field-collected insects. Four sampling sites in the South Island of New Zealand were used to
85 collect second instar larvae of *Costelytra* spp. (Figure 1). These sites are labelled A, B, C and D
86 in Table 1. Collection sites A and B were dominated by exotic plants, while sites C and D were
87 essentially composed of native grasses (Table 1). In the two latter sites, larvae of both species

88 were collected under large patches of native vegetation. These patches were distant enough from
89 exotic vegetation, to ensure that no -or minimal- contact with exotic plants had occurred prior to
90 experiments, given the very low mobility of the earliest larval stages in *Costelytra* spp (Kain
91 1975).

92 Initially, the larvae were placed individually into ice tray compartments with a piece of carrot as
93 food at 15 °C ambient temperature for four days to test for the presence of the endemic amber
94 disease (*Serratia* spp.) according to the protocol of Jackson *et al.* (1993). Healthy larvae were
95 identified to the species level based on the non-invasive methodologies developed by Lefort *et al.*
96 (2012, 2013).

97 *Trifolium repens* (white clover) was grown in a glasshouse (Lincoln University, New Zealand)
98 from seeds (PGG Wrightson Seeds Ltd, Christchurch, New Zealand) in 200 ml of potting mix
99 comprising 60% peat and 40% sterilized pumice stones. Young plants of the native *Poa cita*
100 (silver tussock) were purchased from a native plant nursery in Christchurch, New Zealand. Each
101 plant was carefully transferred from its original pot to a 200 ml pot, filled with potting mix as
102 described above, and was allowed to grow for 2 months prior to the feeding experiment.

103

104 ***Costelytra* spp. feeding preferences – native vs exotic host choice test**

105 The feeding preferences of *C. zealandica* and *C. brunneum* larvae were tested using a three
106 choice olfactometer with native or exotic hosts at 15 °C. The olfactometer comprised of three
107 extended arms, each 120 mm in length and 40 mm in diameter, filled with gamma-irradiated soil
108 (Schering-Plough Animal Health, Wellington, New Zealand) and a 40 x 40 mm central exposure
109 chamber. The larvae were introduced through an aperture in the central chamber. A pot containing
110 either no plant (control pot), white clover, or silver tussock was connected at the end of each arm.
111 Third instar larvae of *C. zealandica* collected from sites B (exotic pasture, n = 35) and C (native
112 grasslands, n = 35) and *C. brunneum* from collection site D (native grasslands, n = 35) were used

113 for this experiment. For each population, the bioassay was replicated seven times, with five new
114 larvae inserted together in the central exposure chamber, in order to mimic the natural clustered
115 distribution of the larvae in the field and to test a greater number of larvae. After 24 hours, pots
116 were disconnected from the olfactometer device, emptied of their contents and larvae were
117 counted. Between each trial, all components of the olfactometer were washed thoroughly with
118 warm water and left to soak in clean water overnight, finally being left to air-dry on a clean
119 counter and reassembled. Results were analyzed with GLMs (family = poisson) using R software
120 (R Development Core Team, 2014). Two separate GLMs were performed: 1) choice (plant) vs no
121 choice (control or no choice) and 2) native host plant vs exotic host plant as response variables, a
122 subset of the choice data. The populations of *C. zealandica* and *C. brunneum* from the different
123 sites were analyzed separately.

124 ***Costelytra zealandica* fitness response on different host plants**

125 Newly moulted third instar larvae of *C. zealandica* collected from sites A (exotic pasture, n = 64)
126 and C (native grasslands, n = 47) were randomly allocated to the two different host plant
127 treatments (white clover and silver tussock). Each larva was kept individually in a 35 ml plastic
128 container containing 50 g of gamma-irradiated soil (as above) and was fed *ad libitum* with roots
129 of white clover or silver tussock. Containers were randomly arranged on plastic trays and kept in
130 an incubator at 15 °C.

131 The fresh weight of each larva was recorded at the beginning of the experiment and after the first
132 six weeks of treatment. The latter corresponded to the most intensive weeks of feeding for the
133 third instar life stage of this species. All measurements were performed on a 0.01 g readability
134 portable digital scale. The experiment was conducted over an additional 9 weeks, to cover the
135 average 15 week duration of the third instar in *C. zealandica*. Survival rates were assessed after
136 this time.

137 Statistical analyses to determine the effect of host plant diet on larval survival were carried out
138 using a Chi-squared test. For each population, an ANCOVA was conducted to analyze the effect
139 of host plant diet on larval growth after 6 weeks while controlling for initial weight. The analyses
140 were performed after exclusion of larvae that died before the end of the sixth week. The Chi-
141 squared test was conducted using R software (R Development Core Team, 2009), while the
142 ANCOVA were performed using the statistical software SPSS v. 20.

143 **Results**

144 ***Costelytra* spp. feeding preferences – native vs exotic host choice test**

145 In the choice test, only *C. zealandica* collected from exotic pastures (population B) showed a
146 preference for the exotic white clover (GLM, $p < 0.01$, Null deviance= 15.04, Residual deviance=
147 4.15) (Figure 2). In contrast, *C. zealandica* collected from native grassland (population C) and *C.*
148 *brunneum* (population D), did not show a preference for either plant species (respectively: GLM,
149 $p = 0.24$, Null deviance= 23.33, Residual deviance= 21.92, and GLM, $p = 0.87$, Null deviance=
150 8.31, Residual deviance= 8.28) (Figure 2).

151 ***Costelytra zealandica* - larval survival and growth on exotic clover or native tussock**

152 The larvae collected from exotic pastures (population A) displayed survival rates over six time
153 higher when fed on clover (33.3 % survival) compared with larvae fed on native silver tussock
154 (5.5 % survival) ($\chi^2 = 4.43$, $df = 1$, $p < 0.05$) (Figure 3).

155 No treatment effect on larval growth was detected for the population from native grasslands
156 (population C) ($F(2,22) = 3.69$, $p = 0.07$) (Table 2), while the larvae from exotic pastures
157 (population A) gained 0.428g (± 0.005 g) when fed on clover for 6 weeks, which was almost twice

158 as much weight compared with larvae fed on native tussock ($F(2,54) = 12.26, p < 0.001$) (Table
159 2), (Figure 4).

160 Discussion

161 This study investigated variation in feeding preferences and fitness response to various hosts. The
162 results corroborate the existence of strong intraspecific variation of the diet breadth of this pest
163 species (Lefort *et al.* 2014). This study also demonstrated similarities between feeding
164 preferences of a population of *C. zealandica* collected from an isolated native habitat with those
165 of the congeneric non-pest species *C. brunneum*. The overall results of this study have provided
166 new insight into the mechanism(s) underpinning the invasion of *C. zealandica* into improved
167 pastures throughout New Zealand.

168 It is important to note that the nutritional value of the roots on which the larvae fed can vary
169 within the same plant in response to soil nutrient distribution and concentration (Grossman &
170 Rice 2012) and possibly results in differential fitness performance in the same population of
171 insect. However, the overall fitness, as measured by survival and growth, of *C. zealandica*
172 collected from exotic pastures was significantly higher on the exotic host plant than on its native
173 host. Inheritance and maternal effects on host choice (Mousseau & Dingle 1991, Mousseau &
174 Fox 1998), where offspring display high fitness performance (Fox 2006) and similar host
175 preferences to their mother (Craig *et al.* 2001), is a possible explanation. Similarly, another
176 maternal effect coined the ‘mother knows best’ hypothesis, which suggests that females tend to
177 oviposit on host plant(s) that can potentially increase their offspring survival (Scheirs *et al.* 2000,
178 Mayhew 2001), can also be a possible explanation, although no evidence supporting this
179 hypothesis has been observed in *C. zealandica* adult beetles (Kelsey 1968, Radcliffe & Payne,
180 1969, Kain 1975).

181 The effects described above are supported by the results of the choice test. In this test, population
182 A, consisting of *C. zealandica* larvae collected from exotic pasture plants on which the
183 population is likely to have fed for several generations, chose exotic clover as the preferred host
184 plant. In contrast, the population of *C. zealandica* collected from their native range did not show
185 any preference in the choice tests and did not perform better on either host. The first observation
186 negates the hypothesis of inheritance and maternal effect on host choice mentioned earlier, since
187 based on this principle, this population would have been expected to prefer its native host (i.e.
188 silver tussock) and have better fitness performance on this plant compared with the exotic host
189 (i.e. white clover). Unlike silver tussock, white clover is a legume, which may partly explain the
190 differences in larval weight gain observed in the *C. zealandica* population collected from exotic
191 pastures. Indeed, because of their bacterial symbiosis resulting in an ability to fix nitrogen
192 (Awmack & Leather 2002), the nutritional value of this family of plants is likely to be higher than
193 that of grasses, such as silver tussock, used as the alternative host in this study. However, this
194 alternative hypothesis does not explain the response of the other *C. zealandica* population
195 studied, which in this case would have been expected to show increased weight gain on clover as
196 well.

197 Based on similar survival rates observed in the two populations of *C. zealandica* used in this
198 study, and because the population collected from native grassland was presumably isolated
199 enough to have not fed on exotic host plants prior to the experiment, it appears that the successful
200 exploitation of an exotic plant by this species is likely a pre-existing ability. Diegisser *et al.*
201 (2009) and Ding & Blossey (2009) suggested that some form of pre-adaptation was required for
202 the exploitation of a novel host plant. The hypothesis of pre-adaptation or phenotypic plasticity in
203 *C. zealandica* is supported by i) the similarity in host choice between larvae of *C. zealandica*
204 collected from native grassland and larvae of the non-pest species *C. brunneum*, and ii) the
205 differential exploitation of exotic pastoral plants by the two species. However, the limited number

206 of replicates for the population collected from native grassland calls for caution in the
207 interpretation of these results.

208 The defence mechanisms employed by the different host plants and their effect on the fitness of
209 the insect species studied would be an interesting aspect to investigate. In a recent review about
210 phytophagous insects and plant defences, Ali and Agrawal (2012) reaffirmed that generalist
211 insects do not master or totally overcome their host defences, but possess ‘general mechanisms’
212 to tolerate an array of those defences. It is possible to observe variations in this tolerance,
213 particularly when the host-range utilised by the insect species is highly diversified and,
214 consequently, when the family of plants have differential evolutionary histories that may have
215 resulted in slight variations in their defence mechanisms. Here, *C. zealandica* may have been less
216 affected by the defences of white clover compared to those of the other hosts or, conversely and
217 as recently shown by Lefort *et al.* (2015), may have benefited from the defences of their host.
218 The latter phenomenon has been observed several times in recent insect-host interaction studies,
219 where the defences of the hosts were artificially triggered and the resulting fitness response of the
220 insects studied unexpectedly enhanced (e.g. Pierre *et al.* 2012, Robert *et al.* 2012).

221 The results of this study support the pre-existence of characteristics that may have contributed to
222 the invasion success of the New Zealand native scarab *C. zealandica* into exotic pastures
223 throughout New Zealand in contrast to its native congener, *C. brunneum* which maintains small
224 populations in native grasslands. Additionally, the differences in feeding preferences between
225 different populations of the pest species *C. zealandica*, seem to confirm recent evidence (Lefort
226 *et al.* 2014) of the existence of distinct host-races in this species. With regard to cryptic species,
227 many studies have highlighted the importance of correct species identification for the
228 accomplishment of successful biological control (e.g. Rosen 1986, Paterson 1991, Silva-Brandão
229 *et al.* 2013). Similarly, we believe that the delineation of host-races in pest species could have
230 vital implications in terms of pest control management and strategies. For instance, caution

231 should be taken before denominating a species as a single entity by employing terms such as
232 “pest species” or “invasive species”, and care must be taken during insect sampling and
233 subsequent identification, particularly when performing bioassays for which the outcome may
234 vary depending on the host-race used. Because the natural feeding behavior of some insects can
235 be modified by laboratory experimentation, we believe that complementary *in-situ* experiments
236 that would allow the incorporation and investigation of the effect of natural environmental
237 variables on the feeding behavior of *C. zealandica*, would be beneficial. Furthermore we strongly
238 encourage further molecular investigations to confirm the possible existence of host-races in *C.*
239 *zealandica*, which would greatly benefit the field of biological control research in New Zealand.

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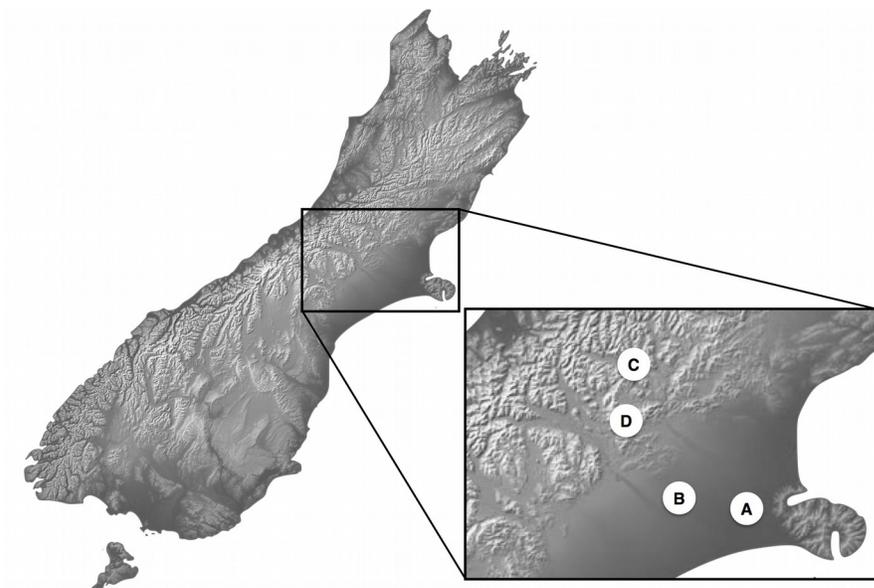
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310 **Tables, figures and legends**311 **Table 1.** General description and location for *Costelytra zealandica* and *C. brunneum* sample sites

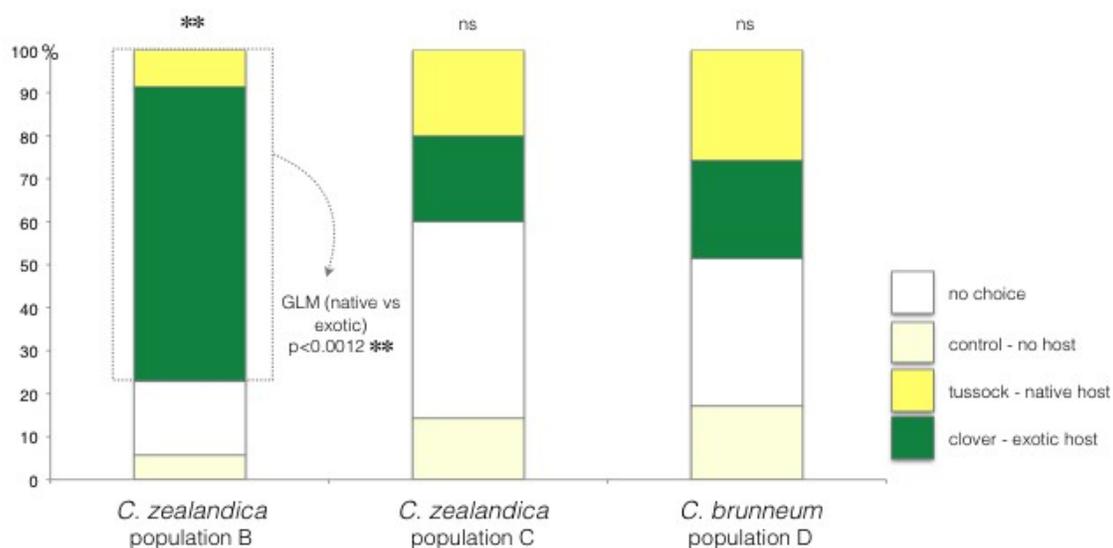
Site	Location	Coordinates	Site description and dominant group of plants	Species sampled and population indexing
A	Lincoln (NZ, South Island)	43°64'04"S 172°47'82"E	mixed exotic ryegrass (<i>Lolium</i> spp.) / clover garden (<i>Trifolium</i> spp.)	<i>Costelytra zealandica</i> (population A)
B	Hororata (NZ, South Island)	43°32'17"S 171°57'16"E	mixed exotic ryegrass (<i>Lolium</i> spp.) / clover dairy pasture (<i>Trifolium</i> spp.)	<i>Costelytra zealandica</i> (population B)
C	Cass (NZ, South Island)	43°02'10"S 171°45'40"E	native tussock grassland (<i>Poa cita</i> over 80% incidence)	<i>Costelytra zealandica</i> (population C)
D	Castle Hill (NZ, South Island)	43°12'20"S 171°42'16"E	native tussock grassland (<i>Poa cita</i> over 80% incidence) close to the margin of beech forest (<i>Nothofagus</i> spp.)	<i>Costelytra brunneum</i>

312 **Table 2.** ANCOVA – effect of different host plant diet on the average weight gain of *Costelytra zealandica* larvae
313 controlling for their initial weight.

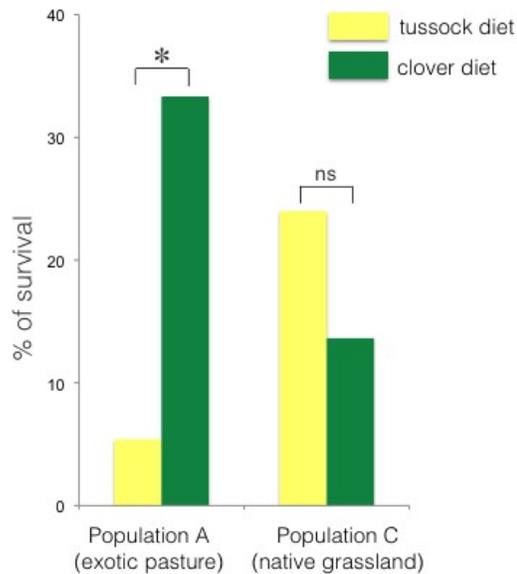
Species (sampling site)	df	F	P values	5% significance level
<i>C. zealandica</i> (population A)				
Treatment	1	12.257	0.001	***
Covariate (initial weight)	1	0.001	0.978	ns
Error	54			
<i>C. zealandica</i> (population C)				
Treatment	1	3.691	0.068	ns
Covariate (initial weight)	1	0.190	0.667	ns
Error	22			



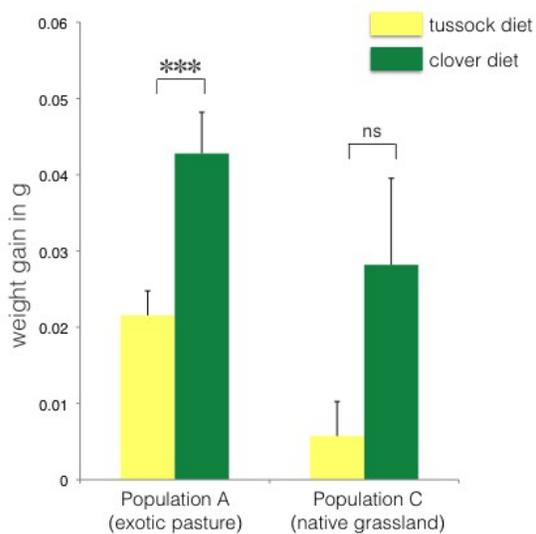
314 **Figure 1.** Location map for *Costelytra zealandica* and *C. brunneum* sample sites.



315 **Figure 2.** Plant choice of larvae of three populations of *Costelytra* in a three-arm olfactometer. With choices of *C.*
 316 *zealandica* population B collected from exotic pastures, *C. zealandica* population C collected from native tussock
 317 grassland, and *C. brunneum* population D collected from native tussock grassland. ** indicates $p < 0.01$ and ns
 318 indicates $p > 0.05$.



319 **Figure 3.** Percentage of larval survival of *Costelytra zealandica* from site A (collected from exotic pasture) and site
 320 C (collected from native tussock grassland) after 15 weeks of feeding on tussock (yellow bars) and white clover
 321 (green bars) host plants. * indicates $p < 0.05$ and ns indicates $p > 0.05$.



322 **Figure 4.** Average fresh weight gain ($\pm 1SE$) of larvae of *Costelytra zealandica* from site A (collected from exotic
 323 pasture) and site C (collected from native tussock grassland) after 6 weeks of feeding on tussock (yellow bars) and

324 clover (green bars) host plants. Pairwise comparisons were performed using an ANCOVA with the initial weight of
325 the larvae as covariate. *** indicates $p < 0.001$ and ns indicates $p > 0.05$.

Table 1 (on next page)

Table 1

General description and location for *Costelytra zealandica* and *C. brunneum* sample sites

Site	Location	Coordinates	Site description and dominant group of plants	Species sampled and population indexing
A	Lincoln (NZ, South Island)	43°64'04"S 172°47'82"E	mixed exotic ryegrass (<i>Lolium</i> spp.) / clover garden (<i>Trifolium</i> spp.)	<i>Costelytra zealandica</i> (population A)
B	Hororata (NZ, South Island)	43°32'17"S 171°57'16"E	mixed exotic ryegrass (<i>Lolium</i> spp.) / clover dairy pasture (<i>Trifolium</i> spp.)	<i>Costelytra zealandica</i> (population B)
C	Cass (NZ, South Island)	43°02'10"S 171°45'40"E	native tussock grassland (<i>Poa cita</i> over 80% incidence)	<i>Costelytra zealandica</i> (population C)
D	Castle Hill (NZ, South Island)	43°12'20"S 171°42'16"E	native tussock grassland (<i>Poa cita</i> over 80% incidence) close to the margin of beech forest (<i>Nothofagus</i> spp.)	<i>Costelytra brunneum</i>

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Table 2 (on next page)

Table 2

ANCOVA - effect of different host plant diet on the average weight gain of *Costelytra zealandica* larvae controlling for their initial weight.

Species (sampling site)	df	<i>F</i>	<i>P values</i>	5% significance level
<i>C. zealandica</i> (population A)				
Treatment	1	12.257	0.001	* * *
Covariate (initial weight)	1	0.001	0.978	ns
Error	54			
<i>C. zealandica</i> (population C)				
Treatment	1	3.691	0.068	ns
Covariate (initial weight)	1	0.190	0.667	ns
Error	22			

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Figure 1 (on next page)

Figure 1

Location map for *Costelytra zealandica* and *C. brunneum* sample sites.

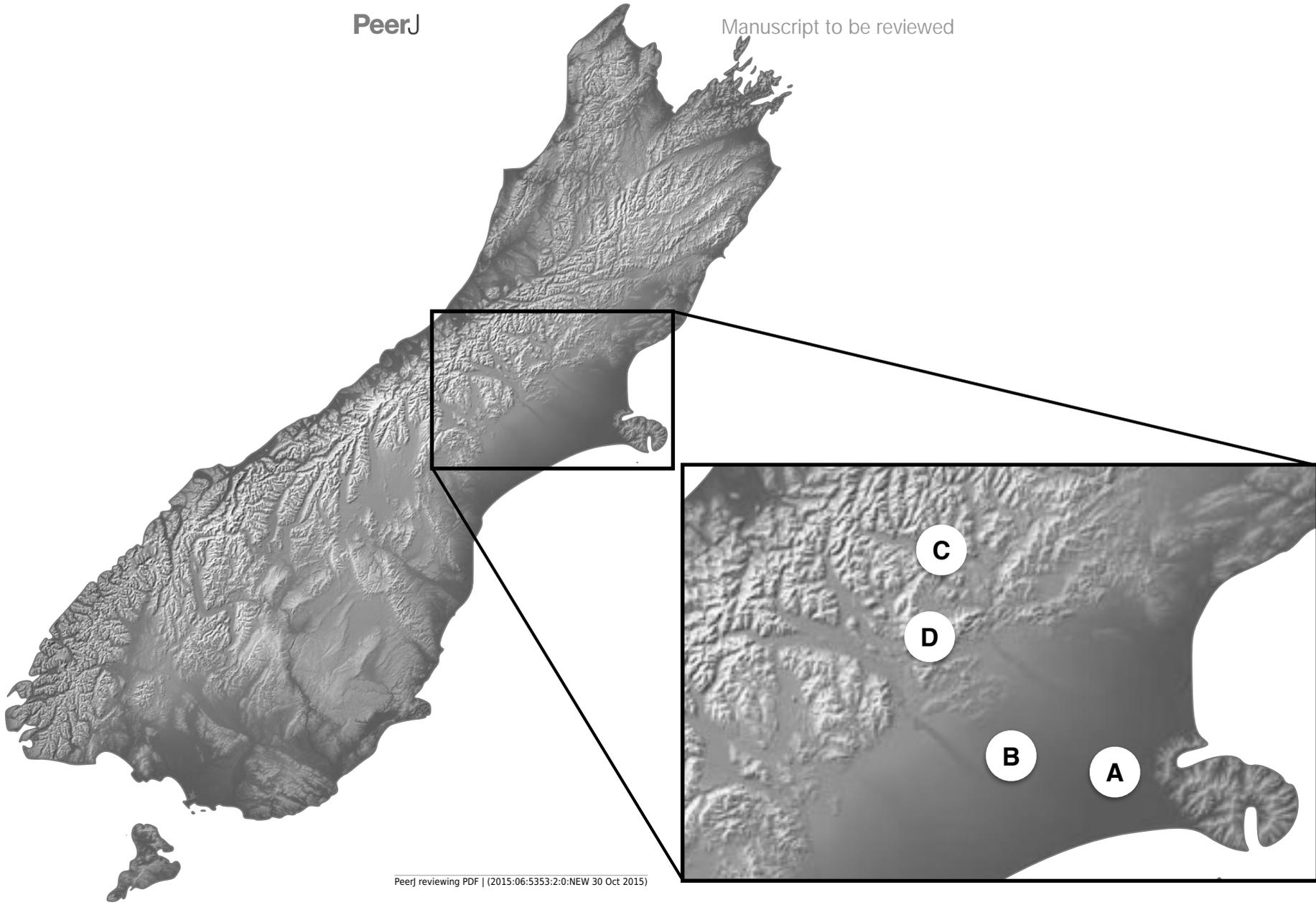


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Figure 2

Plant choice of larvae of three populations of *Costelytra* in a three-arm olfactometer. With choices of *C. zealandica* population B collected from exotic pastures, *C. zealandica* population C collected from native tussock grassland, and *C. brunneum* population D collected from native tussock grassland. ** indicates $p < 0.01$ and ns indicates $p > 0.05$.

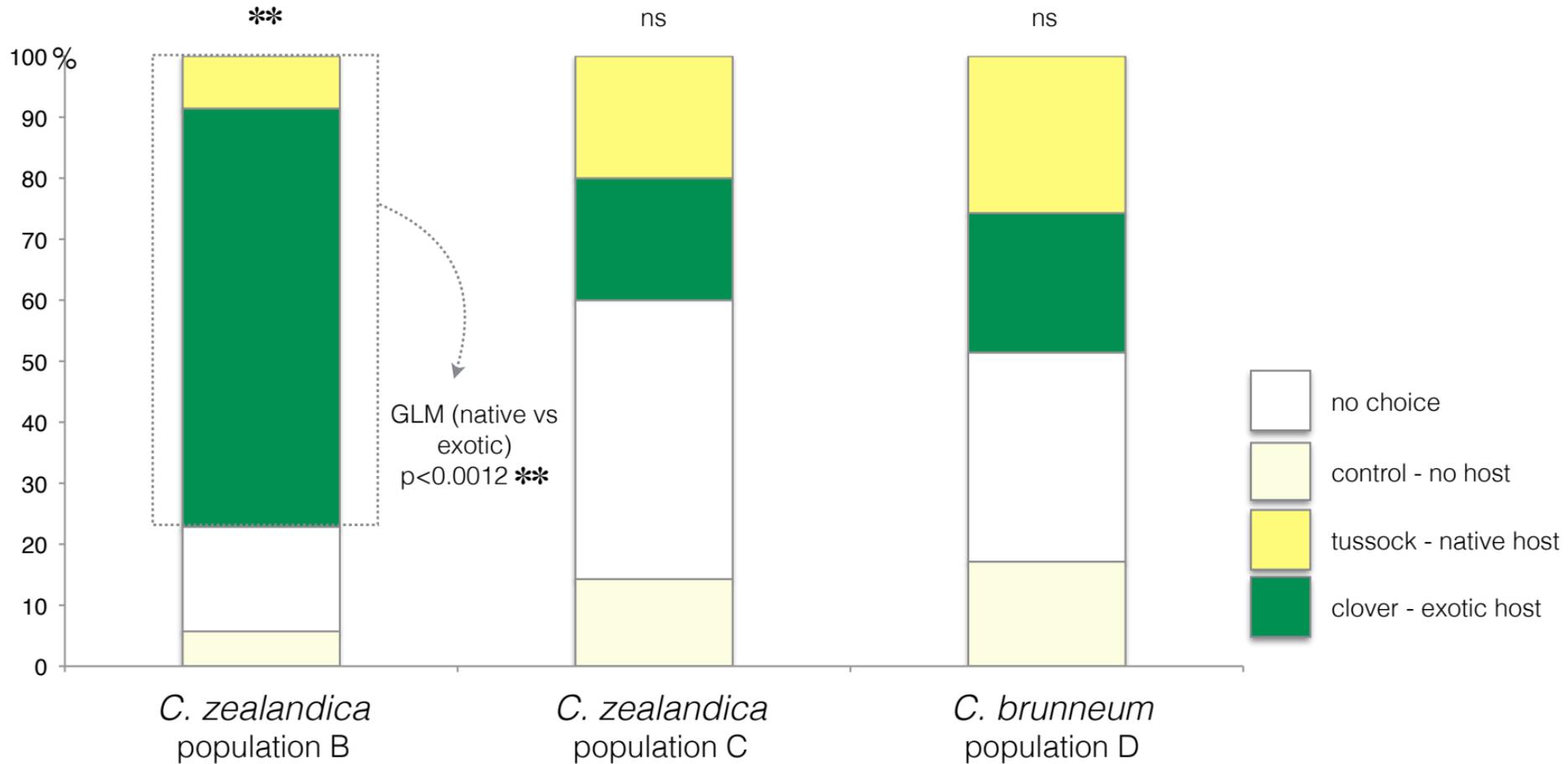


Figure 3 (on next page)

Figure 3

Percentage of larval survival of *Costelytra zealandica* from site A (collected from exotic pasture) and site C (collected from native tussock grassland) after 15 weeks of feeding on tussock (yellow bars) and white clover (green bars) host plants. * indicates $p < 0.05$ and ns indicates $p > 0.05$.

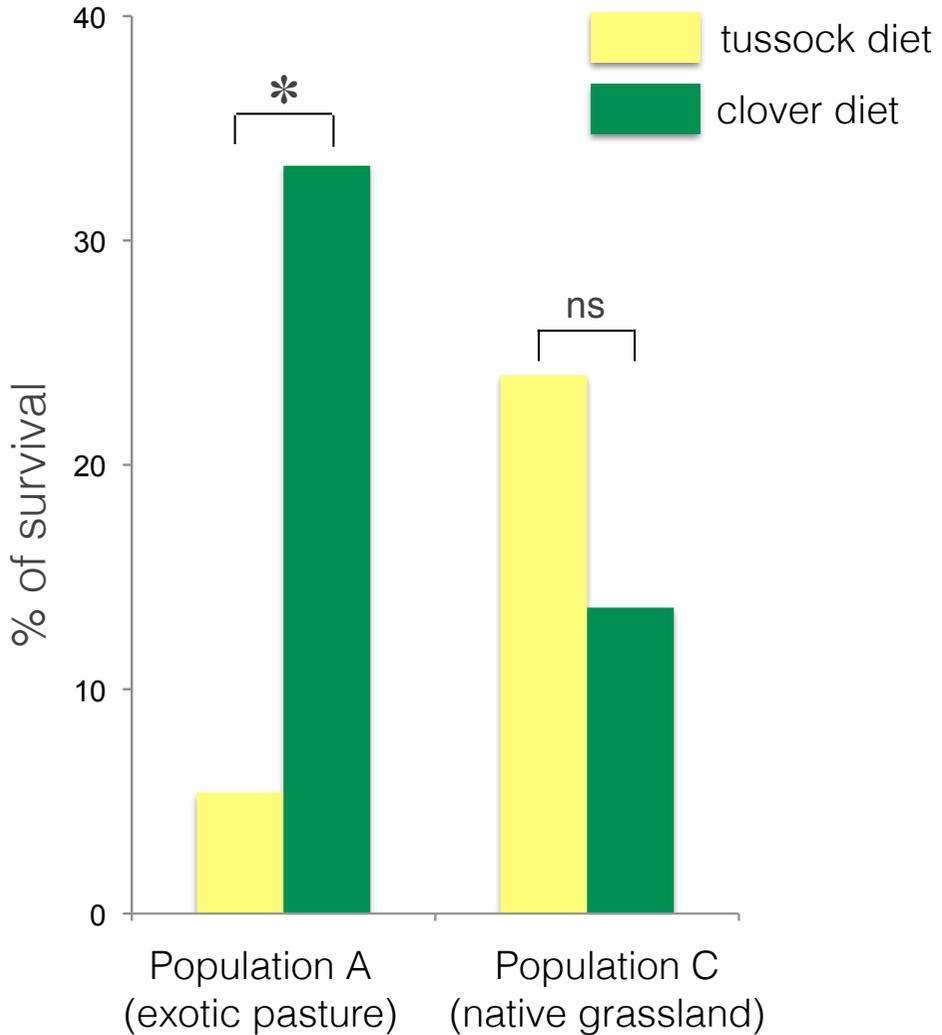


Figure 4(on next page)

Figure 4

Average fresh weight gain (+1SE) of larvae of *Costelytra zealandica* from site A (collected from exotic pasture) and site C (collected from native tussock grassland) after 6 weeks of feeding on tussock (yellow bars) and clover (green bars) host plants. Pairwise comparisons were performed using an ANCOVA with the initial weight of the larvae as covariate. *** indicates $p < 0.001$ and ns indicates $p > 0.05$.

