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A native beetle fond of exotic plants. Characteristics that contribute to invasive success in *Costelytra zealandica* (Scarabaeidae: Melolonthinae).

Abstract

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 the 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 close congeneric species, has not developed such a relationship with these ‘new’ 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 preferences of larvae of both *Costelytra* species were investigated under controlled conditions 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 better 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*. This study suggests the possibility of strong intra-specific variation, in the ability of *C. zealandica* to exploit native or exotic plants, supporting the

38 hypothesis that such ability underpins the existence of distinct host-races in this
39 species.

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42 **Key words:** invasive species, native invader, plant-insect interactions, feeding
43 preferences, New Zealand

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45 **Suggested running head**

46 *A native beetle fond of exotic plants*

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Introduction

By widely replacing native ecosystems with more economically productive land, modern intensive agriculture has often been regarded by ecologists as a driver for substantial biodiversity loss (Robinson & Sutherland 2002, Tilman *et al.* 2002, Foley *et al.* 2005).

Although detrimental for numerous species, anthropogenic modifications creating ‘new’ ecological conditions appear to be beneficial under certain circumstances for some species of the native pool. For instance, it is acknowledged that the high diversity of phytophagous insects partially depends upon evolutionary processes that occur through the action of factors affecting their diet breadth (Gaete-Eastman *et al.* 2004), such as the appearance of a new host plant. Hence, the ecological repercussions of anthropogenic-driven modification(s) on native ecosystems are worth investigating to enhance understanding of the insect invasion process. In addition, the comparison of native and invasive congeners is recognised as a useful approach for identifying characteristics that promote invasiveness (Munoz & Ackerman 2011), even more so, as in this study, when the ‘invasive congener’ is native itself.

In New Zealand, the introduction of exotic pastoral plants has resulted in alteration of the diet of the native coleopteran *Costelytra zealandica* (White) (Scarabaeidae), resulting in the larvae of this endemic insect to feed intensively on the roots of ryegrass and white clover and being ranked as a major economic pest (Pottinger, 1975; Richards *et al.*, 1997). Interestingly and in contrast, *C. brunneum* (Broun), a close congeneric species, is not often found in ryegrass and white clover pastures and remains mostly distributed in native habitats (Given, 1966; Lefort *et al.*, 2012, 2013). Both *Costelytra* species are considered as univoltine organisms (Atkinson & Slay 1994) with three larval stages,

although it is not uncommon to come across individuals that follow a two-year life cycle in the highest and coldest environments of the southern locations of New Zealand, such as Otago and Southland (Stewart 1972, Kain 1975). These two species are sympatric and share similar native hosts, mainly comprising tussock species (Poaceae) commonly found in New Zealand native grasslands (Given, 1966; Lefort *et al.*, 2012, 2013).

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

Material and methods

Insect sampling and plant material

Newly hatched third instar larva, as the most damaging life stage of the invasive species *C. zealandica* and the most intensively feeding life stage in *Costelytra* spp. in general, were used in perform the experiments. Because no protocol exists to produce *Costelytra* spp. offspring under laboratory conditions and all attempts to do so have failed, the best second option was to work with field collected insects. A total of four sampling sites in New Zealand's South island were used to collect second instar larvae of *Costelytra* spp., Lincoln (43°64'04''S 172°47'82''E) and Hororata (43°32'17''S 171°57'16''E), sites A and B, and Cass (43°02'10''S 171°45'40''E) and Castle Hill (43°12'20''S 171°42'16''E),

as sites C and D (Figure 1). Extensive taxonomic assessments of the plants present on each site were not performed. However, the two highly dominant groups of plants present on sites A and B were exotic ryegrass (*Lolium* spp.) and clover (*Trifolium* spp.), while sites C and D were dominated by native tussock (e.g. *Poa cita*) (visually estimated over 80% incidence) with no white clover (*Trifolium repens*) being present. In the two latter sites, larvae of both species were collected under large patches of native vegetation. These patches were distant enough from exotic vegetation, to ensure that no -or minimal- contact with exotic plants had occurred prior to experiments, given the very low mobility of the earliest larval stages in *Costelytra* spp.

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

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

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

The feeding preference of *C. zealandica* and *C. brunneum* larvae were tested using a three choice olfactometer with native or exotic hosts. The olfactometer comprised of three extended arms, each 120 mm in length and 40 mm in diameter, filled with gamma-irradiated soil (Schering-Plough Animal Health, Wellington, New Zealand) and a 40 x 40 mm central exposure chamber. The larvae were introduced through an aperture in the central chamber. A pot containing either no plant (control pot), white clover, or silver tussock was connected at the end of each arm. Third instar larvae of *C. zealandica* collected from sites B (population B from exotic pasture, n=35) and C (population C from native grasslands, n=35) and *C. brunneum* from collection site D (population D from native grasslands, n=35) were used for this experiment. For each population, the bioassay was replicated seven times, with five new larvae inserted together in the central exposure chamber. After 24 hours, pots were disconnected from the olfactometer device, emptied of their content and larvae were counted. Between each trial, all components of the olfactometer were washed thoroughly with warm water and left to soak in clean water overnight, finally being left to air-dry on a clean counter and reassembled. Results were analyzed by Chi-squared test using R software (R Development Core Team, 2009). The significance of the choice of a plant (i.e. white clover or silver tussock) versus no plant (control) and no choice was first tested. In the event that a plant was chosen, the significance of the plant choice itself was also tested, in other words, post hoc follow-up testing was performed on white clover versus silver tussock.

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

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

The experiment was conducted over 15 weeks, which is representative of an average length of the third instar stage of *C. zealandica*, after which all larvae were assessed for survival. At the commencement of the experiment and after the first six weeks of treatment, which corresponded to the most intensive weeks of feeding for the third instar life stage of this species, the weight of each larva was recorded. Statistical analyses to determine the effect of host plant diet on larval survival were carried out using a Chi-squared test. Average larval growth was analyzed by Welch Two Sample t-test for each population, after exclusion of larvae that died before the end of the sixth week. All statistical analyses were conducted using R software (R Development Core Team, 2009).

Results

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

In the choice test, only *C. zealandica* collected from exotic pastures (population B) showed a preference for the exotic white clover ($\chi^2 = 7.88$, $df = 1$, $p < 0.01$) (Figure 2). In contrast, *C. zealandica* collected from native grassland (population C) and *C. brunneum*, did not show a preference for either plant species (Figure 2).

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

In contrast to the larvae from native grasslands (population C), the larvae collected from exotic pastures (population A) displayed significantly higher survival rates when fed on clover (33.3% survival) compared with larvae fed on native silver tussock (5.5% survival) ($\chi^2 = 4.43$, $df = 1$, $p < 0.05$) (Figure 3).

No treatment effect on larval growth was detected for the population from native grasslands (population C) ($t = -1.84$, $df = 13.19$, $p = 0.089$), while the larvae from exotic pastures (population A) gained significantly more weight when fed on clover for 6 weeks compared to when they were fed on native tussock ($t = -3.38$, $df = 40.08$, $p = 0.0016$) (Figure 4).

Discussion

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

Overall fitness, as measured by survival and growth, of *C. zealandica* collected from exotic pastures was higher on the exotic host plant, white clover. Inheritance and

maternal effects on host choice (Mousseau & Dingle 1991, Mousseau & Fox, 1998), where offspring display high fitness performance (Fox 2006) and similar host preferences to their mother (Craig et al. 2001), is a possible explanation. Similarly, another maternal effect coined the ‘mother knows best principle’, which suggests that females tend to oviposit on host plant(s) that can potentially increase their offspring survival (Scheirs *et al.* 2000, Mayhew 2001), can also be a possible explanation although no evidence toward this principle has been observed in *C. zealandica* adult beetles (Kelsey 1968, Radcliffe & Payne, 1969, Kain 1975).

These effects are supported by the results of the choice test, where population A, comprising the pest species *C. zealandica*, collected from exotic pasture plants on which they were likely to have been feeding for several generations, chose exotic clover as the preferred host plant. In contrast, the population of *C. zealandica* collected from their native range did not show any preference in the choice tests and did not perform better on either host. The first observation negates the hypothesis of inheritance and maternal effect on host choice mentioned earlier, since based on this principle, this population would have been expected to prefer its native host (i.e. silver tussock) and have better fitness performance on this plant compared with the exotic host (i.e. white clover). Unlike silver tussock, white clover is a legume, which may partly explain the differences in larval weight gain observed in the *C. zealandica* population collected from exotic pastures. Indeed, because of their bacterial symbiosis resulting in an ability to fix nitrogen (Awmack & Leather 2002), the nutritional value of this family of plants is likely to be higher than that of grasses, such as silver tussock, used as the alternative host in this study. However, this alternative hypothesis does not explain the response of the other *C.*

208 *zealandica* population studied, which in this case would have been expected to show
 209 increased weight gain on clover as well.

210 Based on similar survival rates observed in the two populations of *C. zealandica* used in
 211 this study, and because the population collected from native grassland was presumably
 212 isolated enough to have not fed on exotic host plants prior to the experiment, it appears
 213 that the successful exploitation of an exotic plant by this species is likely a pre-existing
 214 ability. Diegisser *et al.* (2009) and Ding & Blossey (2009) suggested that some form of
 215 pre-adaptation was required for the exploitation of a novel host plant. The similarity of
 216 host choice, observed between *C. zealandica* collected from native grassland and the non-
 217 pest species *C. brunneum*, along with the current difference in exploitation of exotic
 218 pastoral plants by the two species supports the hypothesis of some degree of pre-
 219 adaptation or phenotypic plasticity in *C. zealandica*. The defence mechanisms employed
 220 by the different host plants and their effect on the fitness of the insect species studied is
 221 an interesting question. In a recent review about phytophagous insects and plant defences,
 222 Ali and Agrawal (2012) reaffirmed that generalist insects do not master, and in this sense
 223 do not totally overcome their host defences, but possess ‘general mechanisms’ to tolerate
 224 an array of those defences. It is possible to observe variations in this tolerance,
 225 particularly when the host-range utilised by the insect species is highly diversified and,
 226 consequently, when the family of plants have differential evolutionary histories that may
 227 have result in slight variations in their defence mechanisms. Here, *C. zealandica* may
 228 have been, in terms of fitness, less affected by the defences of white clover compared to
 229 those of the other hosts or, conversely and as recently shown by Lefort *et al.* (2015), may
 230 also have benefited from the defences of their host. The latter phenomenon has been

231 observed several times in recent insect-host interaction studies, where the defences of the
 232 hosts were artificially triggered and the resulting fitness response of the insects studied
 233 unexpectedly enhanced (e.g. Pierre *et al.* 2012, Robert *et al.* 2012).

234 The results of this study support the pre-existence of characteristics that may have
 235 contributed to the invasion success of the New Zealand native scarab *C. zealandica*, into
 236 exotic pastures throughout New Zealand in contrast to its native congener, *C. brunneum*
 237 that maintains small populations in native grasslands. Additionally, the differences in
 238 feeding preferences between different populations of the pest species *C. zealandica*, seem
 239 to confirm recent evidence (Lefort *et al.* 2014) of the existence of distinct host-races in
 240 this species. With regards to cryptic species, many studies have highlighted the
 241 importance of correct species identification for the accomplishment of successful
 242 biological control (e.g. Rosen 1986, Paterson 1991, Silva-Brandão *et al.* 2013). Similarly,
 243 we believe that the delineation of host-races in pest species could have vital implications
 244 in term of pest control management and strategies. For instance, caution should be taken
 245 before denominating a species as a single entity by employing terms such as “pest
 246 species” or “invasive species”, and care must be taken during insect sampling and
 247 identification, particularly when performing bioassays for which the outcome may vary
 248 depending on the host-race used.

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Figures and legends

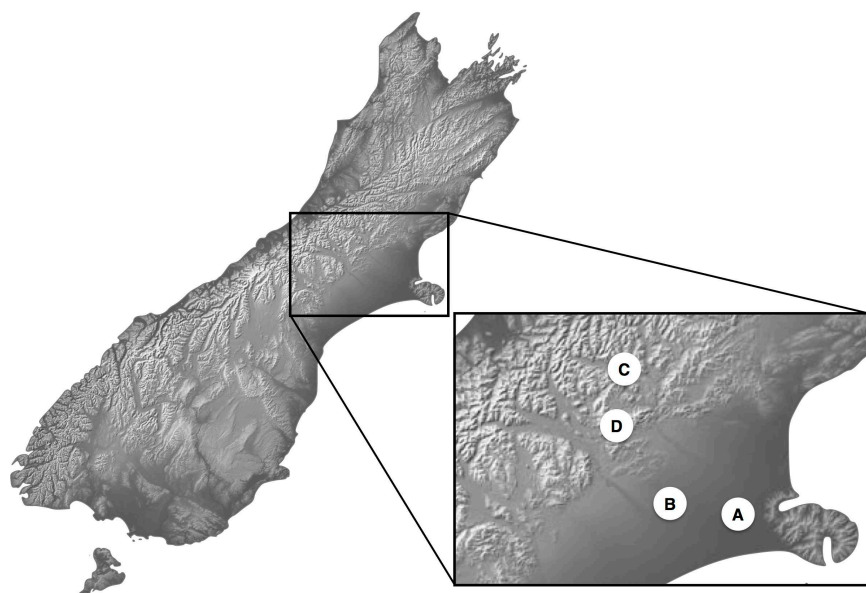


Figure 1. Location map for *Costelytra zealandica* and *C. brunneum* sample sites.

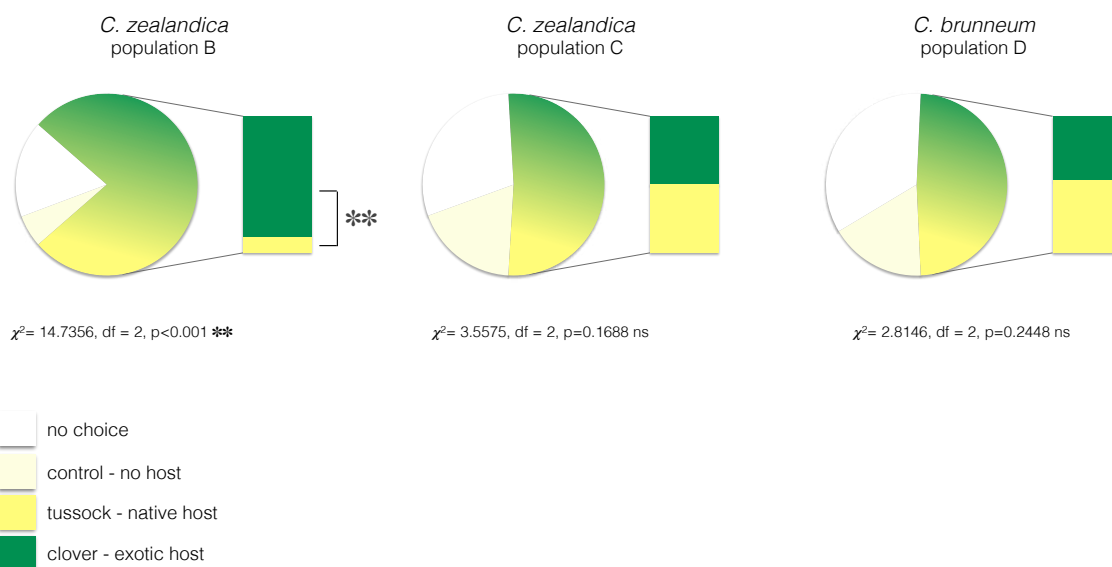


Figure 2. Plant choice of larvae of three populations of *Costelytra* in a three-arm olfactometer. With choices of (a), *C. zealandica* population B (collected from exotic pastures), (b) *C. zealandica* population C (collected from native tussock grassland) and (c) *C. brunneum* population D (collected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

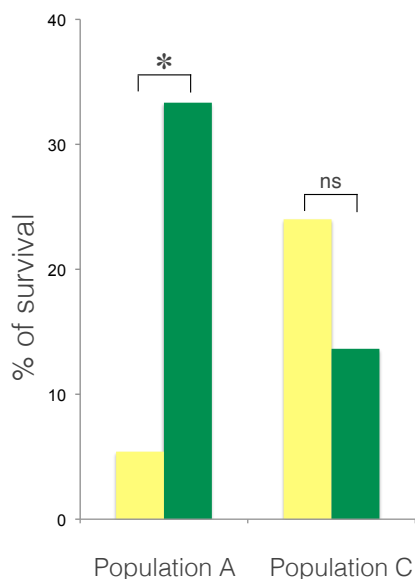


Figure 3. Percentage of larval survival of *Costelytra zealandica* from site A (collected from exotic pasture) and site C (collected from native tussock grassland) after 15 weeks of feeding on tussock (yellow bars) and white clover (green bars) host plants. * indicates $p < 0.05$ and ns indicates $p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

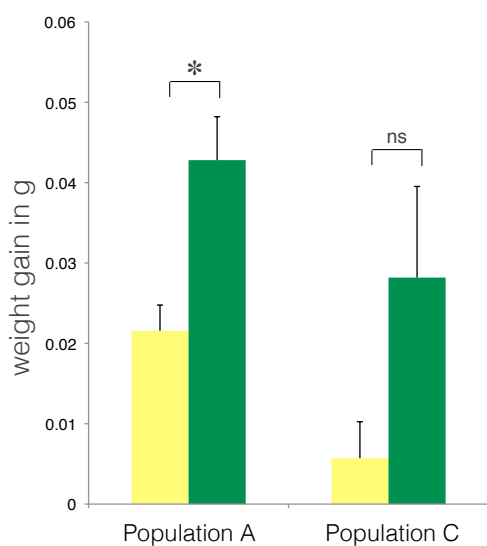


Figure 4. Average weight gain of larvae of *Costelytra zealandica* from site A (collected from exotic pasture) and site C (collected from native tussock grassland) after 6 weeks of feeding on tussock (yellow bars) and clover (green bars) host plants. Vertical bars represent SE. Pairwise comparisons were performed using Welch Two Sample t-test. * indicates $p < 0.05$ and ns indicates $p > 0.05$.