

SHORT COMMUNICATION

Responding positively to plant defences, a candidate key trait for invasion success in the New Zealand grass grub *Costelytra zealandica*Marie-Caroline Lefort^{1*}, Susan P. Worner¹, Michael Rostas¹, Jessica Vereijssen² and Stephane Boyer^{1,3}¹Bio-Protection Research Centre, PO Box 85084, Lincoln University, Lincoln 7647, Canterbury, New Zealand²Plant & Food Research, Private Bag 4704, Christchurch 8140, New Zealand³Department of Ecology, Faculty of Agriculture and Life Sciences, PO Box 85084, Lincoln University, Lincoln 7647, Canterbury, New Zealand

*Author for correspondence (Email: Marie-Caroline.Lefort@lincolnuni.ac.nz)

Published online: 28 July 2014

Abstract: Occasionally, exotic plant introductions lead to the emergence of an invasive insect within its native geographical range. Such emergence could be explained by a pre-adaptation of the insect to break through the defences of the new encountered host. We investigated the fitness responses of two New Zealand endemic scarabs (*Costelytra brunneum* and *C. zealandica*) when given a diet of an exotic pasture species, *Trifolium repens*, whose defences were artificially triggered by the phytohormone jasmonic acid. We found differential fitness responses between the two species when they were exposed to a defence-induced diet. We observed a significant weight increase in the invasive species *C. zealandica* when it was fed with treated roots compared with untreated controls, whereas no significant weight increase was observed in the non-invasive *C. brunneum* compared with the control treatments. Our study suggests that *C. zealandica* has a pre-existing ability to tolerate the defence chemicals of its exotic host and, more interestingly, to benefit from them, which may explain why this species has become a serious pest of pasture throughout its native geographical range.

Keywords: below-ground induced defences; jasmonic acid; Melolonthinae; native invader; pre-adaptation; Scarabaeidae; *Trifolium repens*

Introduction

The reasons why some species become invasive, and other closely related species do not, remain unclear. The established explanations for why a species may become invasive either refer to its intrinsic characteristics, such as its dispersal ability (Rejmanek & Richardson 1996), tolerance limits (Jiménez-Valverde & Lobo 2011) or phenotypic plasticity (Price et al. 2003), or to the various external and/or environmental factors that can contribute to the species becoming invasive (Lefort 2013). The latter include theories about bottom-up and top-down controls (e.g. the novel-weapon hypothesis (Callaway & Ridenour 2004) and the enemy-release hypothesis (Elton 1958). When considering a phytophagous insect reaching the status of a successful invader within its own native range, a number of these established characteristics and theories of invasion success may not be so tenable. For instance, some theories appear to apply solely to plant species (e.g. novel-weapon hypothesis), while others were initially developed for plants and later extended to animal species (e.g. dispersal ability, enemy-release hypothesis). Additionally, the initial introduction stage that defines the process of biological invasion, where a species has to overcome major geographical barriers and/or distances (Valéry et al. 2008), does not necessarily apply to a species transitioning to becoming an invader in its native range following anthropogenic changes on its environment (Lefort 2013). These particular invaders were referred to as 'native invader' by Valéry et al. in 2009, and were thereafter

defined as such by Simberloff and Rejmanek (2011). Hence, the dispersal ability of a species, defined by McDowall (2011) as the relocation of a number of individuals from a place of origin to another location, is of less interest for the study of native invaders. In a similar way, the concept of enemy release is of less interest because the invasive species is not always likely to completely escape its natural enemies (Lefort 2013).

Recently, Carey et al. (2012) urged scientists to investigate why some native/endemic species transition into invaders within their native range. The comparison of a relatively recent emergent pest and a closely related congeneric non-invasive species offers an ideal opportunity to explore the reasons for such transitions and to propose alternatives to established hypotheses about key traits and theories of invasion success. Such a possibility is given by *Costelytra zealandica* (White) and *C. brunneum* (Broun), two endemic New Zealand scarabs (Scarabaeidae: Melolonthinae) that have developed distinctive relationships with *Trifolium repens* (white clover), a temperate pasture species introduced into New Zealand because of its high economic value as livestock forage and a green manure crop (Gillett & Taylor 2001; Badr et al. 2012). The larval stage of *C. zealandica* quickly became a serious pest of white clover (Radcliffe 1971), while *C. brunneum*, although able to feed on the roots of this plant (Lefort 2013), did not. The present study tests the hypothesis that the invasion success of the New Zealand native pest *C. zealandica*, in contrast with *C. brunneum*, might be partially explained by a pre-adaptation to overcome the induced defences of its main introduced host, the

white clover. Induction of plant defences is a general response to herbivory and involves the elicitation of the jasmonic acid (JA) signalling pathway by chewing-biting insects (Karban & Baldwin 1997). An experiment was carried out where larval populations of *C. zealandica* and its congener, the non-pest *C. brunneum*, were fed with the roots of white clover, in which defence compounds were artificially triggered by the phytohormone JA.

Methods

Insects and plants

Third-instar larvae of *C. zealandica* sampled from exotic pastures mainly composed of ryegrass (*Lolium* spp.) and white clover (*Trifolium repens*) (43°32'17" S 171°57'16" E) (Population 1, $n = 60$) and from native tussock grasslands (43°02'10" S 171°45'40"E) (Population 2, $n = 60$), and *C. brunneum* sampled from native tussock grasslands (43°12'20" S 171°42'16" E) (Population 3, $n = 20$) were used for this experiment. Larvae were identified to species level on the basis of their raster morphology (Lefort et al. 2013), and for a few difficult specimens, a rapid diagnostic confirmation was performed using the DNA recovered from their frass (Lefort et al. 2012).

White clover plants were grown from seeds in 200 ml of sowing mix comprising 60% peat and 40% sterilised pumice stones. Three weeks after sowing, pots were treated by direct soil injection of 5 ml of 10 μ M JA (Sigma-Aldrich Chemical Co., St Louis, Missouri), while controls were treated with solvent (0.6 ml of EtOH in 4.4 ml of distilled water). Plants were used 48 h after treatment.

Evaluation of defence-induction in white clover roots

Semi-quantitative assessment of trypsin proteinase-inhibitor induction, used as an indicator of the JA pathway activity, was carried out by radial diffusion assay (Jongsma et al. 1993). Briefly, roots of JA-treated clover and controls ($n = 24$) were rinsed twice with distilled water. Sections (200 mg) of root material from individual pots were homogenised in liquid nitrogen and extracted with 175 μ l buffer (100 mM Tris HCl and 10 mM CaCl₂). The extract was centrifuged for 2 min at 13 000g and 4°C. The supernatant (25 μ l) was transferred to wells made into a gel containing bovine trypsin as substrate. After overnight incubation and staining, trypsin proteinase-inhibitor activity was visible as unstained circular inhibition zones around wells. Zone sizes were compared with those obtained from commercial soybean trypsin proteinase-inhibitor in three concentrations (70 pM, 35 pM and 14 pM). All reagents were obtained from Sigma-Aldrich Chemical Co., St Louis, Missouri. Statistical significance of the presence/absence of trypsin proteinase-inhibitor activity was determined by exact binomial tests using the statistical software R (R Development Core Team 2011).

Costelytra spp. response to increased host defences

Larvae of each population were evenly distributed and randomly allocated to two different feeding treatments. Each larva was kept individually in a 35-ml plastic container containing 50 g of gamma-irradiated soil (Schering-Plough Animal Health, Wellington, NZ), to eliminate any residual organic matter and potential pathogens, as per standard rearing conditions (Lefort 2013; Lefort et al. 2014). Containers were randomly

arranged on plastic trays and kept in an incubator at 15°C. Over a period of 6 weeks, 70 larvae were fed with JA-treated clover roots, freshly chopped to avoid defence induction by the feeding larvae themselves, while another 70 were fed with untreated freshly chopped clover roots. Larvae were fed ad libitum, and their weight and survival rates recorded weekly. The cumulative treatment effects on each population were analysed weekly by one-way analysis of variance (ANOVA) with repeated measures, followed by LSD post-hoc analysis, while statistical analyses on the effect of induced host plant defences on larval survival were carried out using Fisher's exact tests. These statistical tests were conducted with GenStat® version 14.1 (VSN International, Hemel Hempstead) and R version 2.12.1 (R Development Core Team 2011), respectively.

Results

Evaluation of defence-induction in white clover roots

Trypsin-inhibitor activity was detected in all JA-treated clover root replicates while no trypsin proteinase-inhibitor activity, and therefore no defence induction, was detected in the untreated control samples (binomial test, $P < 0.001$). In treated roots, inhibition zone diameters were consistently equivalent to those produced by the standard soybean trypsin proteinase-inhibitor at concentrations between 35 and 70 pM.

Costelytra spp. response to increased host defences

After 6 weeks of feeding on JA-treated clover roots, individuals of both *C. zealandica* populations (exotic pasture and native grassland) had significantly larger biomasses than larvae that were fed with untreated roots (Fig. 1a,b). The treatment effect was significant (ANOVA, $F_{1,57} = 5.744$, $P = 0.020$ and $F_{1,58} = 4.853$, $P = 0.032$, respectively for Populations 1 and 2) with clear differences visible as early as the beginning of the third week (Fig. 1a,b). On the other hand, no treatment effect was detected for the non-pest *C. brunneum* (ANOVA, $F_{1,18} = 0.002$, $P = 0.967$) (Fig. 1c).

No significant treatment effect was detected on the survival of the larvae of both species (Fisher's exact tests, $P = 0.706$, $P = 0.353$ and $P = 1$ for Populations 1, 2 and 3 respectively) (Fig. 2). However, at 10% level of significance, the survival of the pest *C. zealandica* was significantly higher than that of the non-pest *C. brunneum* when fed with either JA-treated clover (Fisher's exact tests, $P = 0.052$ and $P = 0.089$ for Populations 1 and 3, and for Populations 2 and 3, respectively) or with the untreated clover (Fisher's exact tests, $P = 0.085$ and $P = 0.002$ for Populations 1 and 3, and for Populations 2 and 3, respectively) (Fig. 2).

Discussion

Our study showed that, when induced, *Trifolium repens* has the capacity to increase its below-ground defences through the JA pathway, and both populations of the pest *C. zealandica* showed significantly better growth compared with the control treatment when fed with JA-treated white clover roots. In contrast, no treatment effect was observed in the non-pest species *C. brunneum*. The analyses of the survival rates of *Costelytra* spp. larvae revealed that both species were able to avoid the effect of an increase of their host defences. The fact that *C. brunneum* showed a lower

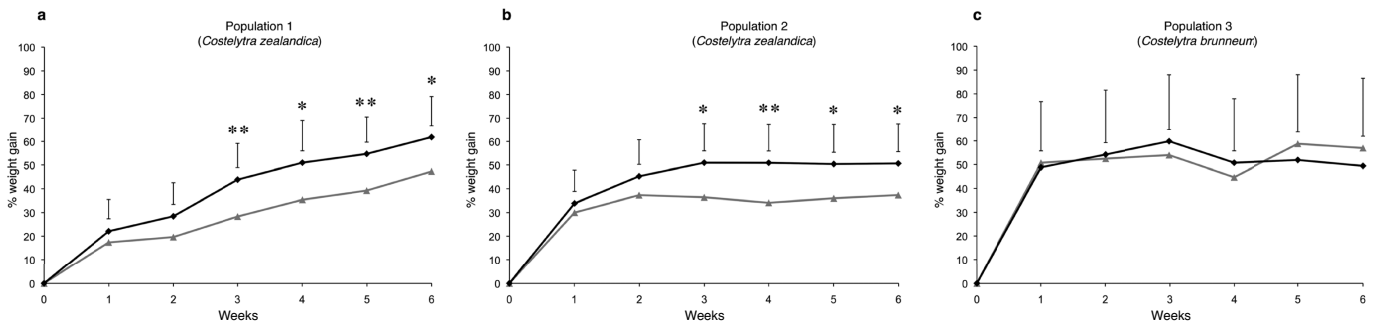


Figure 1. Average larval weight gain (percentage) for *Costelytra zealandica* (a: from exotic pasture; b: from native grassland) and *C. brunneum* (c: from native grassland) in response to a diet consisting of clover roots treated with jasmonic acid (black) or untreated controls (grey). Vertical bars represent 5% Least Significant Difference (LSD).

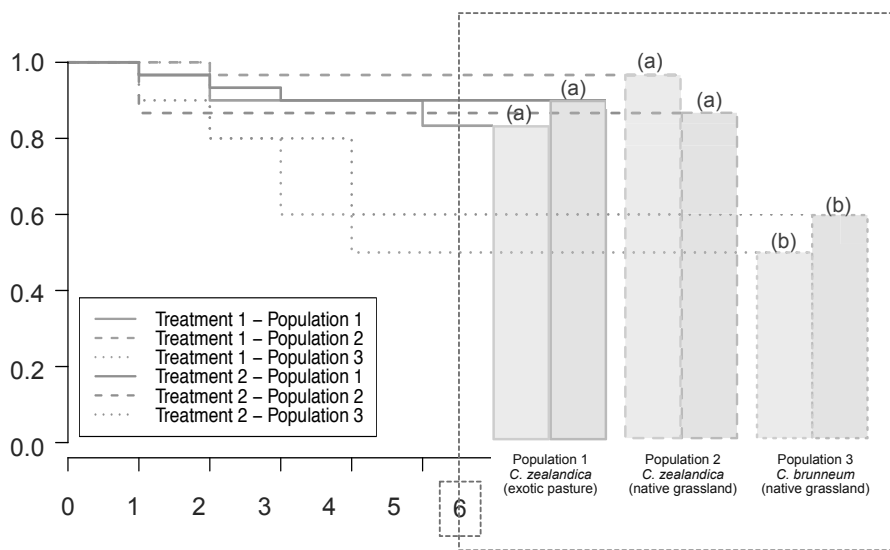


Figure 2. Kaplan–Meier plot of larval survival of *Costelytra zealandica* and *C. brunneum* during 6 weeks of feeding treatment with untreated clover roots (treatment 1) in green or with jasmonic-acid-treated clover roots (treatment 2) in salmon. Right: final survival after 6 weeks of treatment. All pairwise comparisons were performed using Fisher’s exact tests after 6 weeks of treatment. Survival rates with different letters (a vs b) are significantly different (Fisher’s exact tests, $P < 0.05$).

survival rate than its congener *C. zealandica*, both for treated and untreated clover roots, may reflect a certain sensitivity of the species to sampling manipulation, initial health assessment, experimental conditions or monitoring. This higher sensitivity in *C. brunneum* may also be demonstrated by the rapid weight increase during the first week of treatment, once the living conditions for the larvae were eventually stabilised. The lower survival rates were not ascribed to *C. brunneum* feeding preferences, which were similar when exposed to their native hosts or to white clover (Lefort 2013).

Increased fitness as measured by better growth on JA-treated clover was somewhat unexpected for *C. zealandica*; however, recent studies have reported similar responses of insect species to increased plant defences, whether they were triggered by JA application or by insect wounding. For example, Pierre et al. (2012) reported that JA application on turnip plants resulted in increased pupal size of the cabbage fly, *Delia radicum* (Linnaeus), and Robert et al. (2012a) reported that larvae of the Western corn rootworm, *Diabrotica virgifera* Leconte, gained over 30% more weight on maize plants damaged by conspecifics compared with healthy ones.

The detailed chemical nature of insect-induced plant defences remains largely unknown (Jansen et al. 2008) and little attention has been given to defence induction in roots (Erb et al. 2012; Pierre et al. 2012). It is clear, however, that

JA triggers a regulatory cascade that results in the synthesis of numerous primary and secondary plant metabolites (Jansen et al. 2008). Phenolics for example, have been identified among the final products of defence-related genes mediated by the JA defence pathway and are well known for being detrimental to many plant feeding insects (Bernays & Woodhead 1982). Despite this, a significant number of studies have reported counterintuitive positive correlations between high plant phenolic content and high fitness performance in insects (e.g. Bernays & Woodhead 1982; Johnson et al. 2011; Pierre et al. 2012; Robert et al. 2012b). Some suggest that elevated contents of root phenolics rather than being detrimental can have beneficial antioxidant properties for certain phytophagous insects (e.g. Johnson & Felton 2001; Piskorski et al. 2011). Considering that some insects possess the ability to sequester various plant compounds for their own defence (see Jansen et al. (2008) for a review), maybe also, some species, such as *C. zealandica* in this study, have the ability to use certain host defences to their advantage. A possible alternative explanation may rely on the better use of altered primary root metabolite concentrations such as increases in C/N ratio that have been observed in herbivore-damaged clover roots (Murray et al. 1996), rather than on the direct use of secondary host plant compounds by the insect. In this sense, *C. zealandica* may possess an evolutionary advantage that allows it to cope

better with induced host defences than *C. brunneum* and thus allows it to make better use of the high nutrient of these primary metabolites.

While the results here suggest that *C. zealandica* may benefit from host defence induction, it is notable that the population of *C. zealandica* collected from native grasslands also significantly increased weight when fed with the JA-induced 'new' host compared with the insects in control treatments. This population, collected from isolated native grassland, is unlikely to have ever been exposed to exotic white clover before the experiment as suggested by the evolution of distinct host-races in this species (Lefort et al. 2014). Such a result suggests that the ability to avoid the putative detrimental effects of plant defences and seemingly benefit from them has not resulted from an 'arms race' between the insect and its host, but is a pre-adaptation. The ability to avoid detrimental effects of host defence chemicals and to benefit from them has been consistently reported in particular reference to invasive species. For example, high concentrations of total plant phenols appeared favourable to grub development in the common cockchafer *Melolontha melolontha*, an invasive scarab (L. Sukovata, Polish Forest Research Institute, pers. comm.). Similar observations have been reported in other coleopteran pests, such as the vine weevil *Otiorhynchus sulcatus* (Johnson et al. 2011) and the western corn rootworm *Diabrotica virgifera* (Erb et al. 2012) as well as in invasive moths and grasshoppers (Bernays & Woodhead 1982).

Despite previous reports, to our knowledge, this study is the first to hypothesise a link between insect invasion success and a pre-existing ability to overcome and benefit from the defence metabolites of a new host. We expect the preliminary findings of this study to open new research perspectives in the field of invasion ecology and invite researchers to replicate the experiment described in this paper using other invasive/non-invasive species pairs. The confirmation of such a pre-adaptation as a key trait of invasive phytophagous insects, within but also outside their native range, could have important implications for improving pest risk assessment to prevent and predict the potential economic impact of newly introduced phytophagous insects into new areas.

Acknowledgements

The authors thank the Miss E. L. Hellaby Indigenous Grasslands Research Trust, Better Border Biosecurity and the Bio-Protection Research Centre for their financial support. We also thank Richard Townsend and St Andrew's College of Christchurch for granting access to the different insect collection sites.

References

- Badr A, El-Shazly HH, Mekki L 2012. Genetic diversity in white clover and its progenitors as revealed by DNA fingerprinting. *Biologia Plantarum* 56: 283–291.
- Bernays EA, Woodhead S 1982. Plant phenols utilized as nutrients by a phytophagous insect. *Science* 216: 201–203.
- Callaway RM, Ridenour WM 2004. Novel weapons: Invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2: 436–443.
- Carey MP, Sanderson BL, Barnas KA, Olden JD 2012. Native invaders – challenges for science, management, policy, and society. *Frontiers in Ecology and the Environment* 10: 373–381.
- Elton CS 1958. *The cology of invasions by animals and plants*. London, Methuen.
- Erb M, Glauser G, Robert CAM 2012. Induced immunity against belowground insect herbivores- activation of defenses in the absence of a jasmonate burst. *Journal of Chemical Ecology* 38: 629–640.
- Gillett JM, Taylor NL 2001. *The world of clovers*. Ames, IA, Iowa State University Press. 457 p.
- Jansen JJ, Allwood JW, Marsden-Edwards E, van der Putten WH, Goodacre R, van Dam NM 2008. Metabolomic analysis of the interaction between plants and herbivores. *Metabolomics* 5: 150–161.
- Jiménez-Valverde A, Lobo JM 2011. Tolerance limits, animal. In: Simberloff D, Rejmanek M eds *Encyclopedia of biological invasions*. Berkeley, CA, University of California Press. Pp. 661–663.
- Johnson KS, Felton GW 2001. Plant phenolics as dietary antioxidants for herbivorous insects: a test with genetically modified tobacco. *Journal of Chemical Ecology* 27: 2579–2597.
- Johnson SN, Barton AT, Clark KE, Gregory PJ, McMenemy LS, Hancock RD 2011. Elevated atmospheric carbon dioxide impairs the performance of root-feeding vine weevils by modifying root growth and secondary metabolites. *Global Change Biology* 17: 688–695.
- Jongsma MA, Bakker PL, Stiekema WJ 1993. Quantitative determination of serine proteinase inhibitor activity using a radial diffusion assay. *Analytical Biochemistry* 212: 79–84.
- Karban R, Baldwin IT 1997. *Induced responses to herbivory*. Chicago, IL, University of Chicago Press.
- Lefort M-C 2013. *When natives go wild...: why do some insect species become invasive in their native range?* PhD thesis, Lincoln University, Christchurch New Zealand.
- Lefort M-C, Boyer S, Worner SP, Armstrong K 2012. Noninvasive molecular methods to identify live scarab larvae: an example of sympatric pest and nonpest species in New Zealand. *Molecular Ecology Resources* 12: 389–395.
- Lefort M-C, Barratt BIP, Marris JWM, Boyer S 2013. Combining molecular and morphological approaches to differentiate the pest *Costelytra zealandica* (White) (Coleoptera: Scarabeidae: Melolonthinae) from the non-pest *Costelytra brunneum* (Broun) at larval stage. *New Zealand Entomologist* 36: 15–21.
- Lefort M-C, Boyer S, De Romans S, Glare T, Armstrong K, Worner S 2014. Invasion success of a scarab beetle within its native range: host range expansion vs. host-shift. *PeerJ* 2: e262.
- McDowall RM 2011. Dispersal ability, animal. In: Simberloff D, Rejmanek M eds *Encyclopedia of biological invasions*. Berkeley, CA, University of California Press. Pp. 154–159.
- Murray PG, Hatch DJ, Cliquet JB 1996. Impact of insect root herbivory on the growth and nitrogen and carbon contents of white clover (*Trifolium repens*) seedlings. *Canadian Journal of Botany* 74: 1591–1595.
- Pierre PS, Dugravot S, Cortesero A-M, Poinso D, Raaijmakers CE, Hassan HM, Dam NM van 2012. Broccoli and turnip plants display contrasting responses to belowground induction by *Delia radicum* infestation and phytohormone applications. *Phytochemistry* 73: 42–50.
- Piskorski R, Ineichen S, Dorn S 2011. Ability of the oriental fruit moth *Grapholita molesta* (Lepidoptera: Tortricidae) to detoxify juglone, the main secondary metabolite of the

- non-host plant walnut. *Journal of Chemical Ecology* 37: 1110–1116.
- Price TD, Qvarnström A, Irwin DE 2003. The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B – Biological Sciences* 270: 1433–1440.
- R Development Core Team 2011. R: A language and environment for statistical computing, version 2.12.1. Vienna, Austria, R Foundation for Statistical Computing. <http://www.R-project.org/>.
- Radcliffe JE 1971 Effects of grass grub (*Costelytra zealandica* White) larvae on pasture plants. IV. Effect of grass grubs on perennial rye grass and white clover. *New Zealand Journal of Agricultural Research* 14: 625–632.
- Rejmanek M, Richardson DM 1996. What attributes make some plant species more invasive? *Ecology* 77: 1655–1661.
- Robert CAM, Erb M, Duployer M, Zwahlen C, Doyen GR, Turlings TCJ 2012a. Herbivore-induced plant volatiles mediate host selection by a root herbivore. *New Phytologist* 194: 1061–1069.
- Robert CAM, Veyrat N, Glauser G, Marti G, Doyen GR, Villard N, Gaillard MDP, Giron D, Köllner TG, Body M, Babst BA., Ferrieri RA, Turlings TCJ, Erb M 2012b. A specialist root herbivore exploits defensive metabolites to locate nutritious tissues. *Ecology Letters* 15: 55–64.
- Simberloff D, Rejmanek M 2011. *Encyclopedia of biological invasions*. Berkeley, CA, University of California Press.
- Valéry L, Fritz H, Lefeuvre J-C, Simberloff D 2008. In search of a real definition of the biological invasion phenomenon itself. *Biological Invasions* 10: 1345–1351.
- Valéry L, Fritz H, Lefeuvre J-C, Simberloff D 2009. Ecosystem-level consequences of invasions by native species as a way to investigate relationships between evenness and ecosystem function. *Biological Invasions* 11: 609–617.