

Predators vs. alien: differential biotic resistance to an invasive species by two resident predators

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Academic editor: Gregory Ruiz | Received 8 February 2013 | Accepted 12 August 2013 | Published 11 October 2013

Citation: MacNeil C, Dick JTA, Alexander ME, Dodd JA, Ricciardi A (2013) Predators vs. alien: differential biotic resistance to an invasive species by two resident predators. NeoBiota 19: 1–19. doi: 10.3897/neobiota.19.4839

Abstract

The success of invading species can be restricted by interspecific interactions such as competition and predation (i.e. biotic resistance) from resident species, which may be natives or previous invaders. Whilst there are myriad examples of resident species preying on invaders, simply showing that such an interaction exists does not demonstrate that predation limits invader establishment, abundance or spread. Support for this conclusion requires evidence of negative associations between invaders and resident predators in the field and, further, that the predator-prey interaction is likely to strongly regulate or potentially de-stabilise the introduced prey population. Moreover, it must be considered that different resident predator species may have different abilities to restrict invaders. In this study, we show from analysis of field data that two European predatory freshwater amphipods, *Gammarus pulex* and *G. duebeni celticus*, have strong negative field associations with their prey, the invasive North American amphipod *Crangonyx pseudogracilis*. This negative field association is significantly stronger with *G. pulex*, a previous and now resident invader in the study sites, than with the native *G. d. celticus*. These field patterns were consistent with our experimental findings that both resident predators display potentially population de-stabilising Type II functional responses towards the invasive prey, with a significantly greater magnitude of response exhibited by *G. pulex* than by *G. d. celticus*. Further, these Type II functional responses were consistent across homo- and heterogeneous environments, contrary to the expectation that heterogeneity facilitates more stabilising Type III functional responses through the provision of prey refugia. Our experimental approach confirms correlative field surveys and thus supports the hypothesis that resident predatory invertebrates are differentially limiting the distribution and abundance of an introduced invertebrate. We discuss how the comparative

functional response approach not only enhances understanding of the success or failure of invasions in the face of various resident predators, but potentially also allows prediction of population- and community-level outcomes of species introductions.

Keywords

Amphipod, biotic resistance, functional response, invader, predation

Introduction

The biotic resistance hypothesis (Elton 1958), and its later formulation as the diversity-invasibility hypothesis (Tilman 1999), posits that invasive species may fail or suffer reduced success in more diverse communities owing to lack of niche space, increased competition and other antagonistic interactions with native and other resident species (eg previous invaders; Levine et al. 2004). Overall, this hypothesis has received equivocal support, because even though many studies show a negative correlation between invasibility and diversity (Maron and Marler 2007) many others report no association (Havel et al. 2005a, Havel et al. 2005b) or even a positive correlation (Dunstan and Johnson 2004). Further, prey naïveté, rather than reduced diversity in insular systems likely drives higher invasibility and impacts (Cox and Lima 2006).

Allied with the concept of biotic resistance, the enemy release hypothesis posits that escape from enemies (such as predators, parasites, pathogens) might explain higher competitive ability and hence the heightened invasiveness of some introduced species (Torchin and Mitchell 2004). Again, the general utility of this hypothesis has been debated (Colautti et al. 2004, Hatcher et al. 2006, Dick et al. 2010). However, it is well known that native generalist predators can readily consume introduced species (Rodriguez 2006, Carlsson et al. 2009); this has been linked to the reduction in spread, distribution or abundance of invaders, although evidence ranges from circumstantial to clearly demonstrating cause and effect (see Reusch 1998, Monserrat et al. 2005, deRivera et al. 2005, Jensen et al. 2007, Carlsson et al. 2010, Carlsson et al. 2011, Twardochleb et al. 2012, Takizawa and Snyder 2012). Indeed, the presence of resident predators is often invoked as an explanation for geographic variation in the establishment success and post-establishment spread and impact of many introduced aquatic species (Baltz and Moyle 1993, Ward et al. 2008, Marsh-Matthews et al. 2011).

Many such hypotheses in invasion ecology have, however, been recently criticized as being vague, poorly defined and their tests therefore not robust (see Jeschke et al. 2012, Heger et al. 2013, Ricciardi et al. 2013). Heger et al. (2013) in particular show that invasion biology could be improved by its hypotheses being “branched” into “specific and testable hypotheses”, such that robust conclusions can emerge. In this context, we argue that tests of the “biotic resistance hypothesis” (BRH) with respect to predation cannot simply rely on demonstrations that introduced species are preyed on by residents, because predator-prey relationships can clearly be stable (Juliano 2001) or so weak as to have a negligible effect on invasive prey abundance (e.g. Twardochleb et al. 2012). We

thus refine the BRH here to our “predator restriction hypothesis”, support for which requires negative associations between invaders and resident predators in the field and, further, evidence of a predator-prey interaction that can regulate or de-stabilise prey populations. Thus, the utilization of the “functional response” of a predator (the relationship between prey density and consumption rate; Holling 1959, Holling 1966) may allow more rigorous tests of this hypothesis, as functional responses can explain and predict the impact of predators on prey populations (see Juliano 2001, Dick et al. 2013). In particular, it is important to distinguish between the various forms of this relationship owing to differences in potential contributions to prey population stability (Juliano 2001, Murdoch and Oaten 1975, Hassell et al. 1977). The Type III functional response is typified by relatively low prey consumption at low prey densities, and hence may provide prey with a refuge from predation pressure and thus promote population stability (but Type III responses can still strongly regulate prey populations and provide biotic resistance; Twardochleb et al. 2012). This is somewhat contrasted with the potentially population de-stabilising Type II functional response, whereby high proportions of prey are consumed at low prey densities, hence potentially de-stabilising those prey populations (although weak Type II functional responses of resident predators can still allow invader prey to proliferate; Twardochleb et al. 2012). Such an approach can therefore demonstrate that the predatory behaviour of resident species translates into a significant reduction in the invader prey population (such as the local extinction of the invader and disjunct distributions of invader and natives), driven by a clearly limiting or potentially de-stabilising predator-prey relationship (e.g. see also Kushner and Hovel 2006). Further, invasive species encounter many potential resident predators and we require methods to establish the relative role of each in biotic resistance.

Here, we test our hypothesis and compare biotic resistance between two residents in a system where field data indicate that an invader might be restricted in distribution and abundance by the two resident species that are known to prey on the invader. We use comparative functional responses, a methodology that has successfully elucidated the impacts of various predatory invaders on native prey (Bollache et al. 2008, Dick et al. 2010, Kestrup et al. 2011, Dick et al. 2013). Specifically, the North American amphipod crustacean *Crangonyx pseudogracilis* has invaded western European freshwaters, but typically shows a mutually exclusive distribution relative to *Gammarus* spp. amphipods over a range of spatial scales – from large geographical areas of N. America and Europe (Barton and Hynes 1976, Pinkster et al. 1992, J. Holsinger personal communication) to between and within individual river basins (Holland 1976, Cao et al. 1996, Dick 1996, MacNeil et al. 2000). This pattern in Europe is often assumed to be driven by environmental factors (see MacNeil and Dick 2011), but may also be linked to predation of the smaller, mainly herbivorous invader *C. pseudogracilis* by the larger European natives *Gammarus pulex* and *Gammarus duebeni celticus* (Dick 1996, MacNeil et al. 1999, 2000). However, support for our ‘predator restriction’ hypothesis requires demonstration that the interaction between natives and invaders can strongly regulate or drive local extinction of the latter. Demonstration of a strong Type II predatory functional response of the resident predators towards the invasive prey, consistent

with field patterns of invasion, contact and overlap of the invader and residents, would be a powerful test of our hypothesis. Further, however, since functional responses are sensitive to environmental heterogeneity, whereby Type II responses often become Type III responses when moving from homogenous to heterogeneous habitats (Lipcius and Hines 1986, Anderson 2003, Alexander et al. 2012), support for our hypothesis would be enhanced if we find consistency in the type and magnitude of functional responses under varying environmental conditions. In addition to this, we expect field distributions to reflect interspecific differences in the abilities of resident predators to exert biotic resistance towards invading prey; specifically, we predict greater functional responses by those resident species that are associated with greater impacts on invader populations in the field.

We thus tested our ‘predator restriction hypothesis’ by: (1) determining field patterns of negative associations between the invasive N. American amphipod *C. pseudogracilis* and the European amphipods *G. pulex* and *G. d. celticus*, by re-analysing several published field survey data sets; (2) examining these data sets for any apparent differential in biotic resistance of the two resident predators on the invader; and (3) experimentally deriving the type and magnitudes of functional responses of the two resident predators towards the invasive prey, in both homo- and heterogeneous habitats.

Materials and methods

Field patterns of amphipod species distributions

We used data from our past intensive surveys of *Gammarus pulex*, *Gammarus duebeni celticus* and *Crangonyx pseudogracilis* in Ireland and a British Island, the Isle of Man (see Dick et al. 1994, Dick 1996, Dick et al. 1997, MacNeil et al. 2009, 2001). *G. d. celticus* is native in the British Isles, but is restricted in freshwaters (but not brackish waters) by the presence of *G. pulex*, which has become resident and naturalized in Ireland and the Isle of Man for several decades (Dick 1996, Dick et al. 1997). *Crangonyx pseudogracilis* is a North American invader now found throughout many catchments (indicated by the aforementioned surveys). We chose the above data sets on the basis that the river and lake systems concerned had all three species present and that the three species have had access to those systems for several decades. Data were from multiple sites within large water bodies and no isolated sites were used as they may have been unrepresentative (e.g. not all species may have had access by simple chance events). Thus, the absence of any species was not due to any regional pattern of invasion or spread, with each study site having clearly been within the colonization range of all three species. Another criterion was that none of the sites were heavily organically polluted, as *C. pseudogracilis* is more pollution tolerant than *Gammarus* spp. Heavily polluted sites were those classed as ‘poor’ or ‘bad’ on the General Quality Assessment Scheme (GQA) for rivers and canals (MacNeil 2006). We thus eliminated as far as possible any gross environmental determinant of the distributions of the three species in freshwaters,

allowing detection of the influence of inter-specific interactions on the distributions of the species. Since the rivers had been sampled using kick samples, while canals, reservoirs and lakes used a combination of kick, sweep and grab samples, we used only presence/absence data as these were comparable among sites, whereas density was not. We tested the association between the presence/absence of each *Gammarus* species and the presence/absence of *C. pseudogracilis*; we then tested the association between *Gammarus* species identity (i.e. *G. pulex* or *G. d. celticus*) and the presence/absence of *C. pseudogracilis*, all with Chi-square tests of association. We corrected for using the same data twice in the above analyses by the very conservative Bonferroni method; thus, we assign significance at $p < 0.025$ (i.e. $0.05/2$).

Animal collection for experiment

During May 2011, amphipods were collected using a Surber sampler (1mm mesh net) from riffle-pool stretches of rivers on the Isle of Man: *G. pulex* from the Middle River (U.K. ordnance survey grid reference SC 368 755); *G. d. celticus* from the Crogga River (SC 343 728); and *C. pseudogracilis* from the Colby River (SC 222 689). Collecting from locations where only one amphipod species occurred allowed us to mimic initial interspecific contact and invasion in the experiment (Dick et al. 1993). Across all sites, there was little variation in water temperature (11.4–11.8°C), pH (7.0–7.2; Gallen Kampf meter), conductivity (199–231 $\mu\text{S cm}^{-1}$; Dist WP; Hanna Instruments) or BOD₅ ($< 2 \text{ mg l}^{-1}$). The average score per taxon (Armitage et al. 1983) biotic water quality index indicated 'good' to 'very good' biological water quality for all three sites (5.5, 5.3 and 6.1 for the Middle, Crogga and Colby, respectively). Species were maintained separately in aerated stock tanks (25 × 50 × 15 cm deep) at 11.5°C, 13:11 h light: dark (both regimes appropriate for the time of year) with source water, substratum, flora and fauna from their sites of origin (Dick 1996).

Experimental assessment of *Gammarus* spp. predation of *C. pseudogracilis*

Animals were allowed to acclimate (with flora and fauna from their collection sites) for 4 days prior to use in experiments and were killed in warm water immediately after experiments. Similar sized *G. pulex* and *G. d. celticus* were selected to match body lengths for experiments by visual inspection (to reduce potential stress effects incurred by handling) and, following experiments, body lengths (base of telson to base of antennae) were measured under a dissecting microscope and means compared between the species with a t-test. Single males (starved for 24 hours prior to experiments to standardise hunger levels) were presented with *C. pseudogracilis* prey (body length, $3.8 \pm \text{S.E. } 0.3 \text{ mm}$), at 9 prey densities (2, 4, 6, 8, 10, 16, 20, 30 and 40; $n = 3$ per density), in plastic dishes (8cm diameter) with 300 ml of individually aerated stream water (50:50 mix of predator and prey source waters). Each replicate had a new predator ie all

replicates were independent. These densities were realistic, as field densities can reach 1300 individuals m^2 in the Colby River (SC 222 689; MacNeil pers. obs). Replicates were run for both simple (bare container) and complex habitats (washed fine gravel substrate, four glass pebbles and a 5cm strand of washed Canadian pondweed, *Elodea canadensis*), all concurrently. The latter mimics the typical habitat that *Gammarus* and *Crangonyx* species are found in, ranging from streams with gravel substrate to lake shores that also include vegetation. Also, since similar experimental substrate induced a change from Type II to Type III functional responses in another of our amphipod studies (Alexander et al. 2012), its use here gives us confidence that we are providing a realistic opportunity for use of refuge by prey to potentially drive changes in the predator functional response. Replicates were initiated at 17.30 hours and numbers of prey eaten determined at 16 hours; this was the number of prey killed and either wholly or partially consumed. Controls were three replicates of each prey density/habitat type without *Gammarus* predators present. All predator and potential prey individuals were used only once.

Statistical analyses

All statistical analyses were performed using the statistical software R, version 2.14.1 (R Development Core Team 2011). Mean numbers of prey eaten were examined with respect to 'predator species', 'prey density' and 'habitat type' in a three-factor ANOVA (tests of normality (Shapiro-Wilks W -test, $p > 0.05$) and equality of variance (Bartlett's test, $p > 0.05$) indicated raw data were appropriate for such analysis). There are numerous modelling approaches to the assessment of functional responses and choice of model may depend on whether a particular study is mechanistic or phenomenological in approach (Jeschke et al. 2002). Thus, the mechanistic application of parameters such as attack rate and handling time must be approached with extreme caution, or be supported with empirical measurements of parameter estimates (Caldow and Furness 2001, Jeschke et al. 2002, Jeschke and Hohberg 2008). Phenomenological use of these parameters does, however, provide a tool to examine differences in functional response types and parameter estimates in comparative or factorial experiments and this is the approach taken here (see also Alexander et al. 2012, Dick et al. 2013). Thus, in R, logistic regression was used to determine if the *Gammarus* spp. displayed Type II or Type III predatory functional responses, by testing the relationship between the proportion of prey consumed and prey density (Juliano 2001). In this method, a significant negative first order coefficient indicates a decreasing proportion of prey killed as prey density increases, that is, a Type II functional response, whereas a significant positive first order coefficient followed by a significant negative second order coefficient indicates a Type III functional response (Trexler et al. 1988, Juliano 2001). We then fitted Rogers' random equation for data based on experiments where prey numbers deplete in replicates over time and prey individuals are not replaced upon consumption (Juliano 2001):

$$N_e = N_0 (1 - \exp (a (N_e h - T))) \quad (1)$$

where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack constant, h is the handling time and T is the total time available. Owing to the implicit nature of the random predator equation, the Lambert W function was implemented to fit the model to the data (Bolker 2010). Bootstrapping was then used to generate multiple estimates ($n=15$) of the response parameters of attack rate a and handling time h and maximum feeding rate $1/hT$, which were then compared with respect to 'predator species' and 'habitat type' in two factor ANOVAs. As before, data met the assumptions of normality (Shapiro-Wilks W -test, $p > 0.05$) and equality of variance (Bartlett's test, $p > 0.05$)

Results

Field patterns of amphipod species distributions

We had data for 316 field sampling sites in Ireland and the Isle of Man that satisfied our selection criteria to detect interspecific interactions as distinct from gross environmental determinants of resident/invaser species distributions (see above). Where *G. pulex* was absent, *C. pseudogracilis* occurred at 38% of sites, whereas where *G. pulex* was present, *C. pseudogracilis* occurred at a significantly lower proportion of sites (7%; $X^2 = 21.6$, d.f.=1, $p < 0.0001$). Where *G. d. celticus* was absent, *C. pseudogracilis* occurred at 36% of sites, whereas where *G. d. celticus* was present, *C. pseudogracilis* again occurred at a significantly lower proportion of sites (18%; $X^2 = 5.9$, d.f.=1, $p < 0.02$). Further, however, *C. pseudogracilis* occurred at a significantly lower proportion of *G. pulex* sites than at *G. d. celticus* sites ($X^2 = 4.7$, d.f.=1, $p < 0.025$).

Experimental assessment of *Gammarus* spp. predation of *C. pseudogracilis*

No control *C. pseudogracilis* died over the course of 16 hours and therefore experimental deaths were ascribed to predation by *Gammarus*, which were directly observed killing and feeding on *C. pseudogracilis*. This was also evidenced by scattered *C. pseudogracilis* body parts accumulating on the bottoms of containers. There was no significant difference in mean body length between experimental animals of the two *Gammarus* spp. (means (+/- SE) = 15.54 (0.07) and 15.57 (0.08) mm for *G. pulex* and *G. d. celticus* respectively; $t_{106} = 0.4$, NS).

Significantly more *C. pseudogracilis* were consumed by *G. pulex* as compared to *G. d. celticus* ($F_{1,72} = 43.1$, $p < 0.001$; Figs 1a, b), at higher prey densities ($F_{8,72} = 108.7$, $p < 0.001$; Figs 1a, b) and in simple habitat as compared to complex habitat ($F_{1,72} = 12.6$, $p < 0.001$; Figs 1a, b). There was a significant '*Gammarus* spp. \times prey density' interaction effect ($F_{8,72} = 3.1$, $p < 0.01$), reflecting the steeper rise and higher asymptote in prey

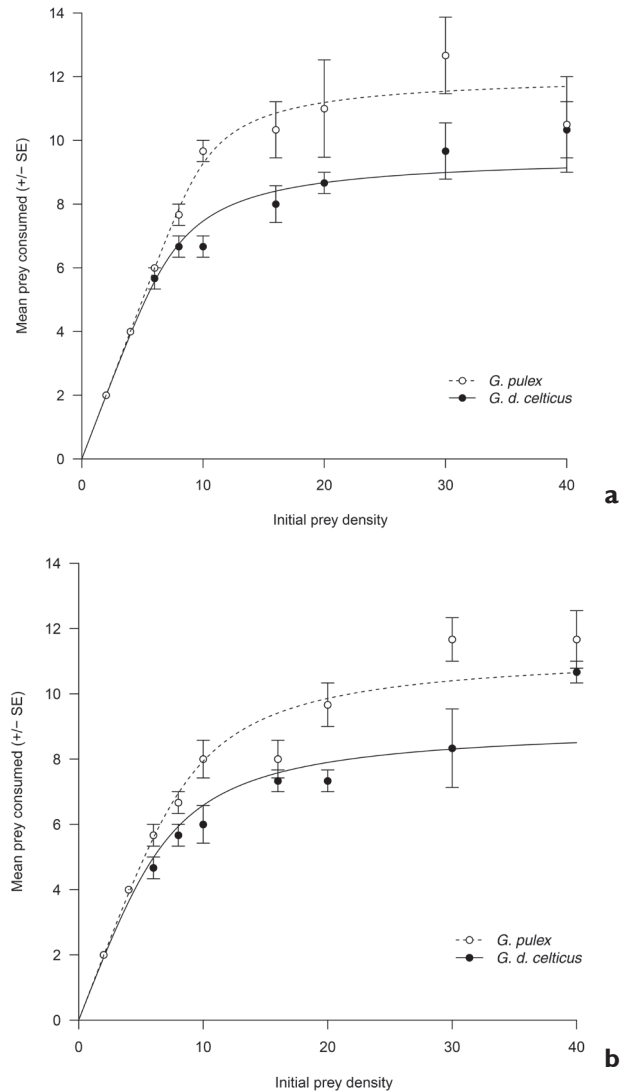


Figure 1. Functional responses of the native European predators *G. pulex* and *G. d. celticus* towards *C. pseudogracilis* prey in **a** simple and **b** complex habitats.

Table 1. Linear coefficients (lc) and significance levels derived from logistic regression analyses of proportion of *Crangonyx pseudogracilis* killed against initial density, with the native predators *Gammarus pulex* and *Gammarus duebeni celticus*, in simple and complex habitats.

Predator species	Habitat type	lc	P	Functional response type
<i>G. pulex</i>	Simple	-0.095	<0.001	II
	Complex	- 0.073	<0.001	II
<i>G. d. celticus</i>	Simple	-0.075	<0.001	II
	Complex	-0.062	<0.001	II

numbers consumed by *G. pulex* relative to *G. d. celticus* as initial prey density increased (c.f. Figs 1a and b).

Both resident *Gammarus* predators exhibited potentially population de-stabilizing Type II functional responses towards the invasive *C. psuedogracilis* in both simple and complex habitats (Figs 1a,b and Table 1). Mean attack rate a was significantly higher for *G. pulex* compared to *G. d. celticus* ($F_{1,56} = 30.6$, $p < 0.001$; Fig. 2a) and significantly higher in simple as compared to complex habitats ($F_{1,56} = 83.4$, $p < 0.001$; Fig. 2a). A significant 'predator species \times habitat type' interaction effect ($F_{1,56} = 4.8$, $p < 0.05$; Fig. 2a) reflects a greater difference in attack rate between the two predator species in simple as compared to complex habitats (Fig. 2a). Mean handling time h was significantly lower for *G. pulex* compared to *G. d. celticus* ($F_{1,56} = 128.1$, $p < 0.001$; Fig. 2b) and significantly lower in simple as compared to complex habitats ($F_{1,56} = 6.8$, $p < 0.05$; Fig. 2b). There was no significant interaction ($F_{1,56} = 0.2$, NS; Fig. 2b). Mean maximum feeding rate $1/hT$ was significantly higher for *G. pulex* as compared to *G. d. celticus* ($F_{1,56} = 157.3$, $p < 0.001$; Fig. 2c) and significantly higher in simple as compared to complex habitats ($F_{1,56} = 8.2$, $p < 0.001$; Fig. 2c). There was no significant interaction ($F_{1,56} = 1.7$, NS; Fig. 2c).

Discussion

The 'biotic resistance hypothesis' (BRH; Elton 1958, Levine et al. 2004) posits that resident species (natives and previous invaders) can constrain the success of invasive species, but the hypothesis does not specify at what stage of the invasion process (see Blackburn et al. 2011) this might occur: do natives resist the introduction, establishment, or spread of the invader? Further, biotic resistance may occur through many mechanisms; although most emphasis has been placed on competition as the mode of resistance (Levine et al. 2004), consumption of invaders by resident species has also been invoked (Maron and Vila 2001, Monserrat et al. 2005, deRivera et al. 2005, Jensen et al. 2007, Carlsson et al. 2009, Carlsson et al. 2010, Carlsson et al. 2011, Twardochleb et al. 2012). However, broad hypotheses such as the BRH require refinement if they are to be specific and testable (Heger et al. (2013)); simply demonstrating the existence of a predator-prey relationship between residents and invaders does not in itself provide evidence of biotic resistance, as predator-prey interactions can be stable (Juliano 2001), predators may not affect the abundance of their prey (e.g. Twardochleb et al. 2012) and predators and their prey can clearly co-exist. More compelling support requires demonstration that resident predators have population-level effects on the invader – as evidenced by, for example, local extinctions of invaders and disjunct resident/invader distributions. Further, demonstrating that the predatory impact of residents can limit an invader, such as by characterising the functional response of residents to invaders (see Twardochleb et al. 2012, Dick et al. 2013), would be compelling evidence of cause and effect. We thus refine the BRH for the purposes of this study to our 'predator restriction hypothesis' and test this in the field and laboratory in a system of two resident predatory amphipods and their invasive prey.

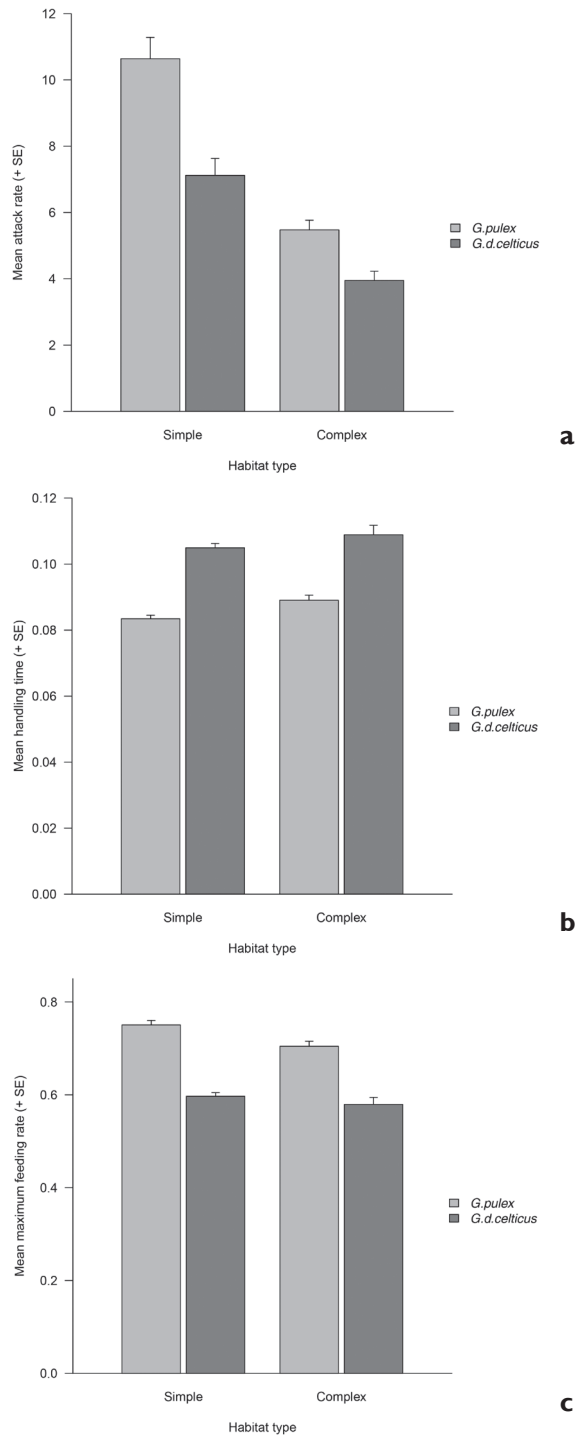


Figure 2. Mean (+SE) **a** attack rate **b** handling time, and **c** maximum feeding rate derived from bootstrapping (n = 15) for *G. pulex* and *G. d. celticus* when habitat structure was simple and complex.

Our survey data sets of the European residents *Gammarus pulex* and *G. duebeni celticus* and the N. American invader *Crangonyx pseudogracilis* reveal that the latter species has strong negative associations with the two former species. All rivers and lakes considered in our analyses (see Dick et al. 1994, Dick 1996, Dick et al. 1997, MacNeil et al. 2009, 2001) have all three species present and the invader has had several decades in which to spread throughout the systems. We can thus assume that *C. pseudogracilis* has had ample opportunity to colonise all sites, despite being absent from the majority of sites where the aforementioned residents are found. There may be some abiotic component of such patterns, whereby *Gammarus* spp. are less likely to be found in heavily organically polluted areas where *C. pseudogracilis* can survive (MacNeil and Dick 2011). However, this cannot explain the disjunct distributions of invader and residents in, for example, stretches of the same lake shoreline or river where water quality is consistent among sample sites, as with our sampling sites selected and analysed here. Indeed, the current study excluded any grossly organically polluted sites and we thus eliminated as far as possible this potential major environmental driver of differing species distributions and associations. Further, during transplantation experiments, *C. pseudogracilis* was able to survive at *Gammarus* sites when the invader was in bioassay tubes that allowed water exchange but protected the invader from contact with the residents (MacNeil et al. 2000), strongly suggesting that biotic rather than abiotic factors limit the spread of the invader.

Our field data also revealed a significantly greater negative association of the invasive *C. pseudogracilis* with the previous invader *G. pulex* as compared to the native *G. d. celticus*. This is fully consistent with our experimental findings of Type II functional responses of both resident predators towards this invader prey, and with the functional responses of *G. pulex* being significantly greater in magnitude, and its higher attack rates, lower handling times and greater maximum feeding rates than *G. d. celticus*. In addition, for both predators, the functional response was clearly and consistently of Type II even in heterogeneous habitat conditions, where prey may often have refuge from predators, leading to a change to more stabilising Type III functional responses (Lipcius and Hines 1986, Anderson 2003, Alexander et al. 2012, in press). We saw no such change in functional response Type from II to III when predator and prey were housed in heterogeneous conditions, as was noted in our similar experimental systems for the native marine amphipod *Echinogammarus marinus* (Alexander et al. 2012, in press). Although predation rate was somewhat reduced overall in heterogeneous conditions (with lower attack rates, increased handling times and hence lower maximum feeding rates), this latter reduction, although statistically significant, was actually rather trivial biologically; for example, the maximum feeding rate for *G. d. celticus* in heterogeneous conditions only dropped to 0.58 prey per hour (p/hr) from 0.59p/hr in homogeneous conditions, and for *G. pulex* the decline was only to 0.70p/hr from 0.75p/hr. A further consideration of the effect of *Gammarus* as predators is that they themselves are subject to predation, for example by fish, and this could alter their functional responses towards prey through trait-mediated indirect interactions (TMIIIs). We have shown that TMIIIs can alter both the shape and magnitude of am-

phipod functional responses in sometimes counter-intuitive ways (eg heterogeneity increased the magnitude of Type III responses; Alexander et al. in press). We thus encourage more studies of the community context within which the biotic resistance hypothesis, and its refinements, are tested and conducted.

Given that Type II predatory functional responses are considered as potentially de-stabilising towards prey populations owing to the increased risk of mortality at low prey densities (Hassell 1978), and have been suggested as potential drivers in local extinctions of prey (Taylor and Collie 2003, Rindone and Eggleston 2011), our experimental and field results are congruent, with the resident predators showing disjunct distributions with the invader, which mostly fails to establish where the residents are present. This is most extreme with regards to the more efficient predator *G. pulex*, which exhibits a significantly greater magnitude Type II functional response and relatively rarely co-exists with the invader. However, we must not only consider the type of functional response, but also the magnitude, because relatively 'low' Type II functional responses – where predation rate is more than offset by prey reproductive rate – could lead to exponential growth of the prey and hence, in the present context, successful invasion (see Twardochleb et al. 2012). On the other hand, relatively 'high' Type II functional responses, where predation rate outstrips prey reproductive rate, may drive invasive prey to extinction (Twardochleb et al. 2012). Thus, the balance of resident predation rate and invader reproductive rate must be examined to further elucidate the likely population consequences of the resident/invader interaction. Female *C. pseudogracilis* produce on average 33 offspring every 22 days at our experimental temperature (see Hynes 1955, Sutcliffe and Carrick 1981), that is, approximately 1.5 offspring per day. *G. pulex* and *G.d. celticus*, even under heterogeneous conditions, can consume 17 and 14 *C. pseudogracilis* per day, respectively. All else being equal, therefore, the resident predator predation rate can clearly outstrip the invader prey reproductive rate, even when the ratio of predator:prey abundance is 1:1 or substantially in favour of the invader. Such ratios are, however, unlikely in an invasion scenario whereby *C. pseudogracilis* attempts to colonise areas where *Gammarus* populations are established at densities measured in the 100s to 1000s per square metre (Kelly and Dick 2005); indeed, *C. pseudogracilis* was only present at sites where *Gammarus* densities were measured only in the 10s of individuals or were absent (see above and Dick 1996). Our field and laboratory results are thus in agreement with the theoretical biotic resistance framework provided by Twardochleb et al. (2012); that is, we have shown a relatively high Type II functional response that is likely to drive invasive prey extinct, and we find no evidence, even in heterogeneous habitat, of a Type III functional response that is more likely to allow invasion. Our negative field associations of predator and prey, with the complete absence of the invader commonly associated with the presence of resident predators, strongly corroborates the laboratory results. Further, it is clear that comparing two resident predators with respect to their functional responses can reveal the mechanism of differential biotic resistance, again corroborated by our field results whereby the predator with the higher functional response exerts more biotic resistance.

Predator exclusion experiments provide compelling evidence for biotic resistance (Robinson and Wellborn 1988, Carlsson et al. 2011); however, it is often unfeasible to manipulate small invertebrate predators for such experiments in the field. Laboratory microcosm studies, although sometimes criticised as to their ecological realism and problems with edge effects are, however, a fruitful route to identifying mechanisms behind the success or otherwise of invasions (see discussion in Dick and Platvoet 2000). The experimental derivation of the types and magnitudes of functional responses of resident predators towards invasive prey can offer a practical alternative to field based studies and still allow understanding and perhaps prediction of the population- and community-level outcomes of invasions in the face of resident predators. We encourage the use of functional response experiments more broadly in invasion biology, where hypotheses require tests of ecological impacts. Thus, for example, we have shown that the invasive ‘killer shrimp’ *Dikerogammarus villosus* has a higher functional response than native gammarid species, consistent with its field patterns of impact on prey (Bollache et al. 2008), while the magnitude of difference in the functional responses of invader and native mysids in the laboratory explained and predicted impacts on prey species in the field (Dick et al. 2013). This method has been used to test the enemy release hypothesis and discovered that, counter to the predictions of this hypothesis, parasitized invasive amphipods had higher functional responses than those unparasitized (Dick et al. 2010). Differential functional responses of natives and invaders towards juvenile heterospecifics have been used to explain invader/native coexistence in another amphipod system in N. America (Kestrup et al. 2011). In addition, some authors, whilst not using comparisons among species, still elucidate the impacts of invaders by deriving their functional responses (Hoof and Bollens 2004, Jones et al. 2011). It should also be noted that the functional response methodology is not restricted trophically or taxonomically, as all consumers utilise resources in quantifiable ways that can be assessed by their functional responses. Finally, methods of assessing functional responses are not restricted to small-scale experiments, since they can be measured in the field (Schenk and Bacher 2002, Smout et al. 2010) and by scat and gut contents analyses (Hoof and Bollens 2004, Middlemas et al. 2006, Jones et al. 2011), making the comparative functional response method widely applicable and achievable.

Conclusions

The success of invading species may be restricted if biotic resistance occurs as a result of predation by resident species. Support for this requires evidence of negative associations between invaders and resident species in the field, in addition to a strongly regulating or de-stabilising predator-prey interaction. We show that two resident predatory amphipods, one native and the other a previous introduction, have strong negative associations with an invasive amphipod prey in the field. Further, our experiments indicate that the resident species both exhibit potentially de-stabilising Type II functional responses towards the invasive prey, in both homo- and heterogeneous environments.

Furthermore, however, the resident predator exhibiting the greater biotic resistance in the field also had the higher functional response in the laboratory. In addition, resident amphipod predation rates are considerably greater than the reproduction rate of the invader, suggesting biotic resistance is likely as predation can clearly outstrip reproduction. We therefore recommend the use of comparative functional response methodologies as an effective way of understanding, as well as potentially predicting, the success and failure of invasions and testing invasion ecology hypotheses.

Acknowledgements

We thank the Natural Environment Research Council, The Leverhulme Trust and the Esme Fairbairn Trust for funding.

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The effect of insect herbivory on the growth and fitness of introduced *Verbascum thapsus* L.

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Academic editor: Ingolf Kühn | Received 13 March 2013 | Accepted 8 August 2013 | Published 11 October 2013

Citation: Wilbur HD, Alba C, Norton AP, Hufbauer RA (2013) The effect of insect herbivory on the growth and fitness of introduced *Verbascum thapsus* L. NeoBiota 19: 21–44. doi: 10.3897/neobiota.19.5123

Abstract

A majority of the plant species that are introduced into new ranges either do not become established, or become naturalized yet do not attain high densities and are thus considered ecologically and economically unproblematic. The factors that limit these relatively “benign” species are not well studied. The biotic resistance hypothesis predicts that herbivores, pathogens and competition reduce growth and reproduction of individual plants and so suppress population growth of non-native species. We explored the effect of insect herbivory and surrounding vegetation on growth and fitness of the non-native biennial plant *Verbascum thapsus* (common mullein) in Colorado, USA. Mullein is widespread in its introduced North American range, yet is infrequently considered a management concern because populations are often ephemeral and restricted to disturbed sites. To evaluate the impact of insect herbivores on mullein performance, we reduced herbivory using an insecticide treatment and compared sprayed plants to those exposed to ambient levels of herbivory. Reducing herbivory increased survival from rosette to reproduction by 7%, from 70–77%. Of plants that survived, reducing herbivory increased plant area in the first year and plant height, the length of the reproductive spike, and seed set during the second year. Reducing herbivory also had a marked effect on plant fitness, increasing seed set by 50%, from about 48,000 seeds per plant under ambient herbivory to about 98,000 per plant under reduced herbivory. Our findings also highlight that the relationship between herbivory and performance is complex. Among plants exposed to ambient herbivory, we observed a positive relationship between damage and performance, suggesting that, as predicted by the plant vigor hypothesis, insect herbivores choose the largest plants for feeding when their choice is not restricted by insecticide treatment. In contrast to the strong effects of experimentally reduced herbivory, we found that cover of other plants surrounding our focal plants explained relatively little variation in performance outcomes. Overall, we found that herbivore-induced impacts on individual plant performance and seed set are substantial, and thus may help prevent this naturalized species from becoming dominant in undisturbed recipient communities.

Keywords

Biotic resistance, competition, common mullein, performance, seed set, insecticide

Introduction

There are over 29 published hypotheses addressing the success of introduced species in their new range (Catford et al. 2009). Support for the different mechanisms is mixed, with experimental investigations offering conflicting results (Colautti et al. 2004, Catford et al. 2009). One potential reason for this discord is that research has largely focused on species that are either dominant members of the community in their novel range or that incur obvious and extensive environmental and economic costs (Hawkes 2007). However, the vast majority of introduced species are not dominant (Williamson and Fitter 1996), and many are considered benign (Lockwood et al. 2007, Dietz and Edwards 2006) or even beneficial (Schlaepfer et al. 2011) in their new range. The bias in research toward species that cause obvious damage may obscure patterns that would be apparent if more data were available from the entire continuum of non-native species, spanning the spectrum from beneficial to detrimental.

To better understand the mechanisms that enable some introduced species to dominate their new communities we must determine which mechanisms prevent other introduced species from doing so (Mack et al. 2000). In the native ranges of plants, herbivores and pathogens can strongly reduce plant performance (Bigger and Marvier 1998; Carson and Root 1999, Maron and Crone 2006; Morris et al. 2007), and in cases this can be paralleled in the introduced range when native generalist herbivores (Parker et al. 2006) or introduced specialist herbivores (e.g. Suckling 2013) suppress plant performance (a component of biotic resistance; Maron and Vila 2001, Levine et al. 2004, Alpert 2006, Catford et al. 2009, Davidson 1993, Olff and Ritchie 1998, Parker et al. 2006). Insect herbivores in general reduce plant performance more than vertebrate herbivores (Bigger and Marvier 1998), and also are used in the biological control of introduced plants. As such, investigating the degree to which insect herbivores mediate the performance of non-native plants may provide a unique perspective for understanding the spectrum of invasiveness among introduced species.

Recent work illustrates the dual roles of herbivory in shaping the outcome of invasions. In a meta-analysis, Hawkes (2007) compared herbivore damage in the native and introduced ranges of plants and found that the degree of invasiveness in the new range was directly correlated to damage from herbivory. Plants classified as noxious weeds (i.e. on the strongly invasive side of the spectrum) had lower levels of herbivory in the new range, while less weedy plants had similar levels of herbivory in the new and introduced ranges. Introduced populations that escape from herbivores may exhibit increased performance relative to their native counterparts, and yet still be suppressed to some degree by herbivores accumulated in the new range (Colautti et al. 2004). Parker et al. (2006), in another meta-analysis, found that generalist herbivores, with a focus on vertebrates, can suppress introduced plants. Despite the fact that insect her-

bivores can have stronger effects than vertebrates (Bigger and Marvier 1998), relatively few studies have experimentally tested the potential of insect herbivores to impose biotic resistance on introduced plants (Colautti et al. 2004).

Our broad goal here is to gain insight into the processes affecting introduced species that do not regularly dominate their recipient communities. Specifically, we explored the effects of insect herbivory on the performance and fitness of *Verbascum thapsus* (common mullein), by reducing insect herbivory using insecticides. Common mullein is an herbaceous biennial that has been present for several centuries in the North America following its introduction from Europe. It is ideal for exploring biotic resistance mediated by insect herbivores: previous research documents shifts in its ecology in the introduced North American range (Alba and Hufbauer 2012), and while it has exhibited marked expansion during its long residency in its North American introduced range, it is not often considered a species of great concern.

Methods

Study system

Mullein is a monocarpic forb with an annual to triennial life cycle (Reinartz 1984), though it is a biennial through most of its range. It is broadly distributed in North America, occurring in all US states and most Canadian provinces (Gross and Werner 1978). However, it is often restricted to disturbed habitats and thus seldom considered a priority for limited management resources. It germinates in the early spring and forms a woolly-leaved rosette in the first year of growth. After overwintering in the rosette stage, plants send up a flowering spike that can reach up to 2 m in height (Baskin and Baskin 1981, Gross and Werner 1978). While the typical growth form is a single spike, particularly large plants and those that incur apical meristem damage may produce several axillary inflorescences (Lortie and Aarssen 2000).

Mullein reproduces purely by seed. Seeds are small (50 to 100 μm in diameter) and plants are quite prolific; a single plant may produce more than 100,000 seeds in its lifetime (Gross and Werner 1978). Seeds can remain dormant in the soil for long periods before germination (Kivilaan and Bandurski 1981). Plants flower from June to September in the western United States, with a few flowers along the stalk opening every day. The flowers last for one day and will self-pollinate when closing if pollination by insects has not already taken place (Gross and Werner 1978).

Mullein populations in the mountain west of the U.S. are more dense and larger, and individual plants are also larger, than are those in the native European range (Alba and Hufbauer 2012). These changes in performance are at least partially evolutionarily based (Alba et al. 2011, Kumschick et al. 2013) and are associated with escape from several specialist herbivores as well as reduced leaf herbivory relative to native mullein (Alba and Hufbauer 2012; Alba et al. 2012). These patterns suggest that introduced populations have in part escaped natural enemies and may experience weakened top-down regulation.

Reductions in pathogen abundance may well contribute to enemy escape (Alba, Hufbauer, Norton, personal observations). For example, mullein plants grown in a common garden environment in their native range (Czech Republic) experienced high seedling mortality and persistent infection over the lifetime of most plants from an as yet unidentified leaf spot fungus. In contrast, plants growing in a parallel experiment in the introduced range (Colorado) showed no signs of infection (Alba and Endriss, personal observations).

Despite evidence for enemy release at the biogeographic scale, substantial herbivory by generalists as well as co-introduced specialists can occur in introduced populations (Alba and Hufbauer 2012). Indeed, at sites in Colorado, mullein plants can lose up to 25% of their leaf tissue to generalist grasshoppers and caterpillars that have incorporated the weed in their host range (Alba, personal observation). Additionally, two co-introduced herbivorous insect species are found in North America. *Gymnetron tetrum* Fabricius (synonym *Rhinusa tetra*), a specialist weevil, is found throughout the introduced range of mullein. The larvae develop in the maturing seed capsules and consume a majority of seeds within before emerging (Salisbury 1942, Reinartz 1984). A second specialist (*Haplothrips verbasci* [Osborn]) is more common in the introduced range than in the native range (Alba and Hufbauer 2012). Thus, even though escape from enemies may contribute to larger populations and individuals in the introduced range relative to the native range, herbivory still has the potential to reduce performance in the introduced range.

Experimental design

To evaluate the effects of herbivory on performance of common mullein, we experimentally reduced herbivory on plants in the field using insecticides. We imposed two main treatments: reduced herbivory (insecticide) and ambient herbivory (water). Additionally, we added a set of no-water control plants part way through the experiment. We evaluated whether reduced herbivore damage increased survival to reproduction, performance during the first growing season (rosette area, leaf number, and biomass), performance in the second growing season (plant height, inflorescence length) and performance when protected from herbivory during both growing seasons (plant height, inflorescence length and seed production).

The experiment took place at a site in Loveland, CO (40°22'29"N, 105°13'32"W, elevation 1650 m) with a history of substantial disturbance, including being used as a staging area for a large construction project and for grazing. The site is currently owned by Larimer County and is maintained as open space. This site was chosen along with two additional sites (which were lost during severe hail storms in the early stages of the study) for three main reasons: the sites had vegetation representative of other areas in the foothills where common mullein is present, land managers were willing to stop controlling common mullein and other weeds for the duration of the study (i.e. no herbicides, mowing or cutting would take place), and finally, we were able to get permission to spray insecticides as the sites received relatively little foot traffic. Plants along 12 transects were marked with a raised nail and metal tag, and assigned randomly to one of the two main

treatments: reduced herbivory (insecticide) or ambient herbivory (water). We started with 551 plants in April 2009. This comprised both overwintered rosettes, which we followed until they bolted ($n = 126$) and first-year rosettes ($n = 425$), a subset of which we harvested after the first summer ($n = 70$), and the rest of which were followed through their entire lifecycle ($n = 355$). In spring 2010, we started tracking an additional 42 plants along the same transects, which received neither insecticide nor water. Table 1 summarizes sample sizes for the first-year rosettes that were then followed for two seasons.

Treatment application

Plants were sprayed with either an insecticide solution or water every two weeks during the growing season using a 5-gallon (18.9-liter) Solo backpack sprayer. We investigated whether the water added in the ambient herbivory treatment increased plant performance by comparing those plants to the additional 42 plants that received no treatment.

The first insecticide application was a mixture of esfenvalerate, (33112 Insecticide Concentrate, Bengal Products, Inc. 3.48% active ingredient esfenvalerate) which has been shown to have no or little effect on plant growth (Root 1996) and spinosad, (Lawn and Garden Spray with Spinosad, Green Light Company, 0.5% active ingredient spinosad). Spinosad is particularly effective against Thysanoptera, and thus was chosen to target the specialist thrips *H. verbasci*. We used a recommended rate of 0.0015% esfenvalerate and 0.0076% spinosad in water.

We used Bayer Advanced Dual Action Rose & Flower Insect Killer Concentrate with active ingredients imidocloprid (0.72%) and beta-cyfluthrin (0.72%) for all subsequent herbivory reduction treatments. We switched because we anticipated getting more effective control with this systemic insecticide. We first tested whether it affected mullein growth, and observed no effects (Appendix), a finding supported by Williams et al. (2010). The insecticide was diluted and applied at a rate of 0.0028% of imidocloprid and 0.0028% beta-cyfluthrin in water solution.

Herbivore damage

To evaluate the effectiveness of the insecticide in reducing herbivory and to gather data for inclusion in subsequent analyses, we estimated leaf herbivory experienced by each plant every month during both growing seasons. Herbivory was scored from low to high (0 to 4) following Lewis et al. (2006): 0 = no damage; 1 = minimal damage with no more than about 5% of any leaf damaged; 2 = minimal damage plus some leaves with 5–10% damage; 3 = 10–50% damage on multiple leaves, but fewer than half of all leaves affected; 4 = at least half of all leaves with 10–50% damage, and multiple leaves with more than 50% damage.

The specialist seed predator *G. tetrum* consumes nearly all seeds in locules it infests within a given fruit capsule (Salisbury 1942), substantially reducing seed set (Reinartz

Table 1. Sample sizes, mortality, and proportion mortality for the plants (first year rosettes) followed for two growing seasons. Formulas reference the first column of letters to indicate how calculations were done.

	Sample size or proportion	Reduced Herbivory	Ambient	Totals
a	Start of experiment Spring 2009	207	218	425
b	Fall 2009 Harvest ¹	36	34	70
c	Fall 2009 Performance data collection ²	175	169	344
d	Summer 2009 Mortality	27	39	66
e	Proportion dead Summer 2009 ($d/[a-b]$)	0.16	0.21	
f	Overwintering Mortality	2	5	7
g	Overwinter missing (presumed dead)	8	7	15
h	Proportion dead overwinter ($([f+g]/[a-b-d])$)	0.07	0.08	
i	Number Spring 2010 ³	133	133	266
j	Summer 2010 Mortality	3	4	7
k	Proportion dead Summer 2010 (j/i)	0.02	0.03	
l	Bolted 2010	128	122	250
m	Did not bolt	2	7	9
n	Total mortality ($d+f+g+j$)	40	55	95
o	Total proportion dead ($n/[a-b]$)	0.23	0.30	
p	Proportion that did not reproduce ($([m+n]/[a-b])$)	0.25	0.34	

¹ Harvest of 70 of the first-year rosettes, fall 2009, to measure biomass.

² Number of plants for which data on number of leaves and rosette area were recorded in year 1. These included plants on which biomass was measured. Some plants were missed, and thus, this column plus summer mortality does not sum to exactly the same number as at the start.

³ This is the number of plants starting in the second season. It is approximately equal to the number at the Start of Experiment minus (Fall 2009 Harvest + Summer 2009 Mortality + Overwintering Mortality). (Not exactly due to unintended things like incorrectly applied treatments)

1984). Our insecticide treatment was focused on leaf herbivory, but we did also spray inflorescences when they developed. Thus, we also evaluated our ability to reduce seed predators at the end of the 2010 season by comparing seed capsule attack rates between treatments (see *Plant Performance 2010*). We measured weevil attack at 5 locations along each inflorescence ($n_{\text{ambient}} = 60$, $n_{\text{reduced}} = 45$), starting 5 cm from the top, and then dividing the rest of the inflorescence into 5 intervals. At each interval, all of the seed capsules within 2 cm were counted and then dissected to determine if weevils were developing inside.

Plant cover

Cover of plants surrounding target individuals may negatively affect growth via competition, or alternatively may be associated with higher quality sites and greater plant growth. To take these types of processes into account, we estimated the percent cover of plant functional groups and bare ground directly adjacent to each mullein plant. We

used a digital camera (Nikon D90, AF-S Nikkor 18-105 mm 3.5-5.6G ED lens, automatic setting), placed 68 cm above the ground, to photograph cover quadrats during the first week of June in both 2009 and 2010. For small first-year plants, we centered a 30 × 30 cm frame around each plant and estimated cover within the frame, excluding the mullein plant itself. For larger second-year plants, we placed a 15 × 30 cm frame adjacent to the plant, in each of the four cardinal directions. The cover photos were processed using SamplePoint according to the methods described in Booth et al. 2006.

Plant Performance 2009

At the beginning of the growing season, before treatment, we estimated the area of first- and second-year rosettes (from measurements of diameter in both directions) and estimated levels of herbivore damage on the leaves. For first-year rosettes, at the end of the growing season, we collected information on survival and growth of each plant. For these plants ($n = 344$ after summer mortality) we measured rosette area, counted the number of leaves, and took a final estimate of herbivore damage for the year. A subset of these plants ($n = 70$) was harvested to obtain biomass data. We removed plants at their base and oven dried them to constant weight before weighing them to the nearest 0.1 g.

Most second-year plants that started as overwintered rosettes in the spring of 2009 bolted that year, and were harvested as they senesced in mid-September 2009. For each plant we measured plant height, inflorescence length (from the first seed capsule at the base of the reproductive stalk to the top of the stalk), and number of branches.

Plant Performance 2010

In April of 2010 we identified all first-year plants that had overwintered successfully ($n = 266$) and resumed treatment regimes. At the end of the growing season we measured plant height, main stem inflorescence length, and total inflorescence length (i.e., including axillary branches). Additionally, on a subset of plants, we measured seed capsule density and the number of seeds per seed capsule as described below. From these measurements, we calculated the total number of seed capsules per plant, and extrapolated to the total seed set per plant.

Seed capsule density. We measured the density of seed capsules on all of the 259 plants that survived to produce inflorescences greater than 5 cm in length ($n_{\text{ambient}} = 107$, $n_{\text{reduced}} = 110$). We used this cut-off, because seed capsules in the top 5 cm of the inflorescence were smaller and denser than the rest of the inflorescence, and often did not contain successfully developed seeds, and thus were not representative of the inflorescence as a whole. On the plants for which we measured seed capsule density, we counted all seed capsules in a 20 cm length of inflorescence, starting 5 cm from the top. When the inflorescence was shorter than 25 cm, the top 5 cm was still not counted, but all the remaining seed capsules were.

Number of seed capsules. We calculated the number of seed capsules per plant by multiplying the seed capsule density by the total length of the inflorescence.

Number of seeds per seed capsule. To determine the average number of seeds produced per capsule, we collected individual seed capsules from a subset of plants ($n_{\text{ambient}} = 24$, $n_{\text{reduced}} = 22$). We removed seed capsules at equal intervals along the inflorescence for a total of 7 seed capsules per plant. Seed capsules were only collected if the capsule was not yet opened and thus had its full complement of seeds. Each seed capsule was placed in its own envelope. We counted out and weighed 50 seeds from each capsule, then weighed all of the seeds in the capsule. The number of seeds per capsule was then estimated by dividing total mass by mass per seed. Sample size was reduced from 7 from each of the plants to one or two, due to weevil infestation.

Statistical analysis

Herbivore damage

To assess the effectiveness of the insecticide treatment in reducing chewing herbivory, we used the MIXED procedure in SAS 9.2 (SAS, 2012). We evaluated whether herbivory in the first month of the season was comparable across treatments to confirm that there was not an unintentional bias in herbivory at the outset of the experiment. Then we evaluated treatment effectiveness in reducing herbivory by evaluating annual average herbivory. We used the average across months because it had the best explanatory power for the plant performance analyses described below (according to comparisons of AIC, analyses not shown). Treatment was included in these models as a fixed effect and transect as a random effect. We used the GLIMMIX procedure (SAS 9.2) to evaluate whether treatment altered the proportion of seed capsules with weevils, using the events/trials syntax, and a logit link.

Effect of water treatment on plant performance

To evaluate the effects of water addition from insecticide treatments on plant growth, we used a mixed model to compare plants that had received the water-only treatment (ambient herbivory) to the untreated plants. We included treatment as a fixed effect and transect as a random effect. We performed separate ANOVAs for each of the response variables, which included plant height, total inflorescence length, and seed capsules per plant.

Effect of reduced herbivory on plant performance

We used PROC GLIMMIX in SAS 9.2 with a binomial error distribution (alive vs. dead) to test for differences in survival among treatments for the plants treated for both

growing seasons. The model included treatment as a fixed effect and transect as a random effect. Covariates (additional fixed effects) included initial plant size, vegetative cover, and average herbivore damage. While the insecticide treatment was effective at reducing insect attack (see results below), it did not prevent it all together, and levels of herbivore damage were variable within each treatment. Thus, it was also important to include the measure of herbivory.

To evaluate the effects of herbivory on the performance of mullein that survived, we created mixed models in JMP. For first-year plants (measurements taken at the end of the first growing season), response variables were final rosette area, number of leaves per rosette, and rosette biomass. The model included treatment (ambient vs. reduced herbivory), initial rosette area in 2009, cover of surrounding vegetation, average herbivore damage through the season, and the interaction between herbivore damage and treatment as fixed effects and transect as a random effect. For second-year reproductive plants (measurements taken at the end of the experiment), the response variables analyzed using this model included plant height, inflorescence length, number of seed capsules per plant and number of seeds per capsule. The model was the same as for the first-year plants, except that cover data for both years and average herbivory over both years were included. As plants were located along transects, we also checked for serial correlation in the data (spatial correlation along one dimension). There was no evidence for such autocorrelation, thus number along transect was not included in any models (analyses not shown). For several analyses, the Satterthwaite approximation was used in calculating degrees of freedom, as it does not assume variances are equal across sample groups. This can result in non-integer degrees of freedom.

For the above models, we log transformed initial and final area of rosettes, total inflorescence length, and number of seed capsules, to improve the normality of the residuals. All data presented have been back-transformed for ease of interpretation.

Results

Herbivore damage

Damage did not differ between the plants assigned to the two treatments prior to the start of the experiment ($\text{mean}_{\text{ambient}} = 1.0$, $\text{mean}_{\text{reduced}} = 1.0$, $F_{1,528.3} = 0.03$, $P = 0.87$), indicating no bias in treatment assignments at the outset. Subsequent insecticide application significantly reduced average herbivory in 2009 ($\text{mean}_{\text{ambient}} = 1.4$, $\text{mean}_{\text{reduced}} = 1.1$, $F_{1,527} = 27.59$, $P < 0.0001$). Patterns of damage were similar in the second year. In the spring of 2010, the first damage estimates did not differ between treatment groups ($\text{mean}_{\text{ambient}} = 0.6$, $\text{mean}_{\text{reduced}} = 0.7$, $F_{1,255.7} = 0.50$, $P = 0.48$), but rates of herbivory were low at that time. By the second month, levels of leaf herbivory had increased overall, but were significantly lower in the insecticide treated plants relative to the water controls, and this continued to be the case until the experiment was terminated in the fall of 2010 ($\text{mean}_{\text{ambient}} = 1.8$, $\text{mean}_{\text{reduced}} = 1.0$, $F_{1,259.8} = 1.06.1$, $P < 0.0001$). Insecticide

treatment reduced attack by the seed-feeding weevil *G. tetrum* ($F_{1,96}$, $P < 0.0001$) from 24% in water controls to 20% in the insecticide treated plants.

Effect of water treatment on plant performance

Plants in the ambient herbivory (water-only) treatment did not differ from untreated (i.e. no water) controls with respect to plant height ($F_{1,155} = 0.3454$, $P = 0.56$), total inflorescence length ($F_{1,157} = 0.4545$, $P = 0.50$), or seed capsules per plant ($F_{1,147} = 0.0053$, $P = 0.94$).

Effect of reduced herbivory on plant performance

Plant survival

Reducing herbivory over both growing seasons significantly increased survival by 7% (from 70% to 77%, $F_{1,323} = 8.1$, $P = 0.005$). Interestingly, neither initial plant size nor percent cover of surrounding vegetation altered survival rates.

Area, leaf number, and biomass of first-year rosettes

At the end of the first growing season, plants in the reduced herbivory treatment had rosettes with area 17% larger than plants in the ambient herbivory treatment ($F_{1,332.7} = 5.1$, $P = 0.025$, Fig. 1a, Fig. S1a). Despite their greater area, they had comparable numbers of leaves ($F_{1,333.7} = 0.31$, $P = 0.57$, Fig. 1b, Fig. S1b) and biomass ($F_{1,62} = 0.25$, $P = 0.62$, Fig. 1c, Fig. S1c). In both the reduced and ambient herbivory treatment, herbivores chose larger plants, generating a positive relationship between herbivory and rosette area across treatments ($F_{1,333.7} = 4.6$, $P = 0.03$). Final rosette area ($F_{1,336.9} = 14.3$, $P = 0.0002$), number of leaves ($F_{1,336.9} = 30.2$, $P < 0.0001$), and biomass ($F_{1,61.2} = 4.43$, $P = 0.04$) were all positively related to initial area, but were not influenced by cover of other plants (final area $F_{1,336} = 2.60$, $P = 0.11$; number of leaves per plant $F_{1,330.7} = 1.7$, $P = 0.19$; biomass $F_{1,63} = 0.03$, $P = 0.86$).

Growth and reproductive output of bolted plants

Experimentally reducing herbivory increased performance relative to the ambient herbivory controls in the bolting plants. This was true for plants treated for only their second growing season (2009 bolting plants, see supplementary Tables S2a, b) and for those treated for both growing seasons (2010, Tables S3a–d). Specifically, reducing herbivory in only the second growing season (plants that bolted in 2009) increased plant height 8.8% (14 cm, Fig. 2a) and total inflorescence length 12.8%

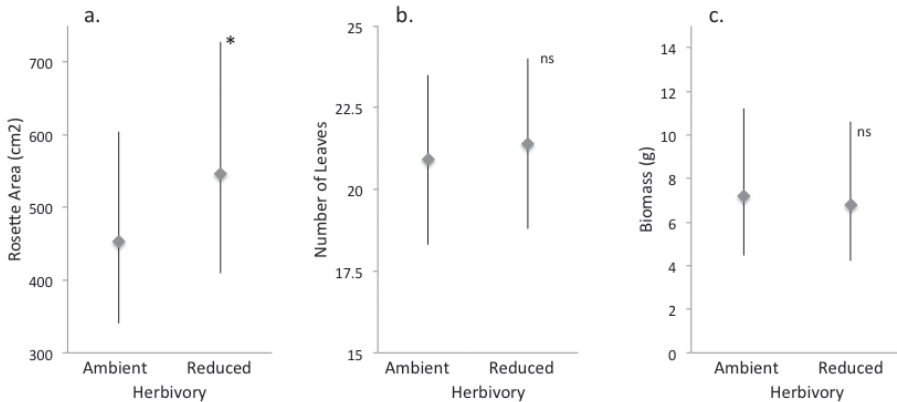


Figure 1. Effect of ambient and reduced herbivory on size of rosettes of *Verbascum thapsus* at the end of one field season of treatment (the plants' first growing season). Panels show **A** final rosette area **B** number of leaves and **C** biomass. Values are model means (backtransformed as appropriate) \pm 1.96SE. A single asterisk indicates a significant treatment difference at $P < 0.05$; ns = not significant. See Tables S1a–c for details, and Figure S1a for a box-and-whisker plot provided for data visualization.

(6.5 cm, Fig. 2b), though the latter difference was only marginally significant. For the plants treated in only their second season, initial rosette area (measured as an overwintered rosette in spring 2009) was associated with taller plants and longer inflorescences (Tables S2a, b) and percentage cover of other plants was associated with shorter plants but this did not change inflorescence length (Tables S2a, b). Greater plant height and inflorescence length was associated with higher average herbivory levels (Tables S2a,b), a pattern discussed further below. For plant height, there was a significant interaction between average herbivory and treatment, such that insect herbivores fed more on larger plants experiencing the ambient herbivory treatment than on smaller ones, but no such pattern was found for plants under reduced herbivory.

Reducing herbivory over two growing seasons increased plant height by 15.6% (19.1 cm, $F_{1,235.3} = 10.99$, $P = 0.001$, Fig. 3a), and increased total inflorescence length by 37.8% (8.1 cm, $F_{1,236.5} = 15.7$, $P < 0.0001$, Fig. 3b). Reducing herbivory also increased the density of seed capsules (number per cm of inflorescence from 7.3 to 8.7, $F_{1,207} = 9.1$, $P = 0.003$). Thus, combining the effects on inflorescence length and capsule density, the total number of seeds per plant was increased a substantial 49% from about 48,000 seeds per plant under ambient herbivory to about 98,000 per plant under reduced herbivory ($F_{1,203.2} = 22.6$, $P < 0.0001$, Fig. 3c). The number of seeds per seed capsule remained comparable between treatments (grand mean of 451, $F_{1,37.8} = 1.6$, $P = 0.21$).

For most performance traits, cover of other plants had modest effects while initial rosette area (in spring of 2009) had substantial effects (Tables S2 and S3). Additionally, there was a significant interaction between average herbivore damage and treatment for most responses due to a positive relationship between herbivory

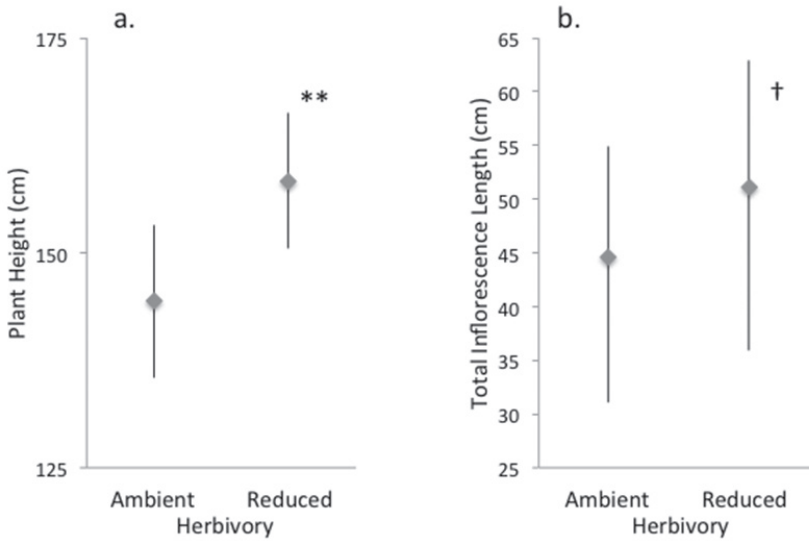


Figure 2. Effect of ambient and reduced herbivory on characteristics of bolting plants after one field season of treatment (the plants’ second growing season). Panels show **A** plant height and **B** inflorescence length. Values are model means (backtransformed as appropriate) $\pm 1.96SE$. Two asterisks indicate a significant treatment difference at $P < 0.005$; † indicates $P < 0.10$. See Tables S2a, b for details, and Figure S1b for a box-and-whisker plot provided for data visualization.

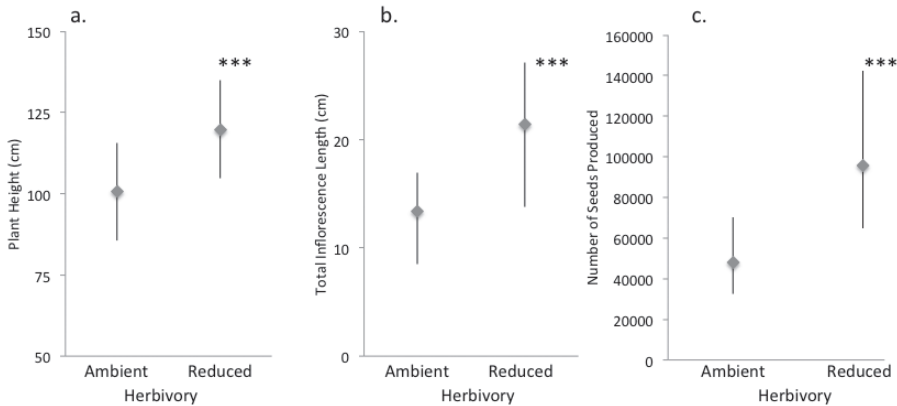


Figure 3. Effect of ambient and reduced herbivory on performance after two field seasons of treatment. Panels show **A** plant height **B** inflorescence length, and **C** seed production of those plants that reproduced. Values are model means (backtransformed as appropriate) $\pm 1.96SE$. Three asterisks indicate a significant treatment difference at $P < 0.0001$. See Tables S3a–d for details, and Figure S1c for a box-and-whisker plot provided for data visualization.

and plant performance for ambient herbivory (water control) plants and no relationship between herbivory and plant performance for reduced herbivory plants (Tables S2 and S3).

Discussion

To elucidate the role that native herbivores and plant competitors serve in resisting invasion, it is necessary to expand current work in invasion biology to include species that are not particularly dominant in their new range. Our work adds to the sparse experimental information available for non-native plants in the middle of the spectrum between benign and strongly detrimental. It also lends insight into the importance of herbivory in shaping various aspects of performance, including lifetime fitness, of plants belonging to this under-studied group.

Our data reveal that herbivory can reduce the performance of mullein at several stages during its life cycle. This evidence for biotic resistance by herbivores present in the recipient community contrasts with the fact that, at a biogeographic scale, mullein in the introduced range incurs significantly less chewing damage than mullein in its native range, and is attacked by fewer types of specialists (Alba and Hufbauer 2012). The duality of these patterns illustrates that while reductions in enemy pressure often manifest at the biogeographic scale, it cannot be assumed that enemy release translates into a fitness advantage over co-occurring native plants in the introduced range (Colautti et al. 2004). Indeed, our data indicate that over the course of mullein's long residence time in North America (approaching 400 years), resident chewing herbivores have successfully incorporated it as a food source, as is the case for other introduced species (e.g., *Junonia coenia*, *Euphydryas editha* and *E. phaeton* feeding on introduced *Plantago lanceolata* in North America; Bowers 1991; Bowers et al. 1992; Singer et al. 1993). Most of the feeding damage we observed was caused by generalist grasshoppers, while across the native European range comparatively few grasshoppers have been observed to feed on mullein (Alba and Hufbauer 2012). It should be noted that, due to the hailstorms mentioned above, our study was conducted at a single location, and thus further investigation is needed to evaluate the degree to which this finding holds across mullein's introduced range. However, the identity of the herbivores present at the site, as well as the damage levels we observed, are representative of populations sampled across a broad portion of the introduced range (Alba et al. 2013; Alba and Hufbauer 2012). Below we discuss the biological relevance of herbivore-induced reductions in performance at different developmental stages.

Effect of reduced herbivory on plant performance

Because mullein is semelparous and cannot spread vegetatively, the most effective form of biotic resistance would prohibit survival to reproduction. This was observed for common mullein, with plants experiencing less herbivory having a 7% higher chance of survival overall. Further, this difference in survival was detected even though reduced herbivory plants were attacked, albeit at a lower level.

Increased performance associated with reduced herbivory was apparent even in a single growing season. First-year rosettes experiencing reduced herbivory had larger area than those exposed to herbivory. Larger overwintering rosettes in turn produced taller plants

in the next season and eventually produced more seed per plant. This finding illustrates that even a partial release from herbivore pressure during the rosette stage can have a significant impact on plant fitness. The importance of rosette size in determining the reproductive output of mullein has also been illustrated in introduced populations growing in the eastern U.S. Gross (1980) reported that first-year rosettes had to be at least 6 cm in diameter in order to successfully overwinter and reproduce. Additionally, the probability that a given mullein plant would die decreased, while the probability that it would flower increased, with increasing rosette size (Gross 1981). In the mesic environment where Gross (1980) worked, she found that interspecific competition from co-occurring natives imposed strong biotic resistance against mullein in the old-field habitat where these studies occurred. In contrast, we found that surrounding vegetation was not associated with the performance of mullein rosettes (when crowding by competitors could critically affect light availability), and only weakly associated with some performance metrics in bolting plants. These results suggest that herbivory imposes stronger resistance than competition in at least some habitats, although competitive interactions have traditionally been invoked as the major source of biotic resistance both generally (Levine et al. 2004) and for mullein (Gross 1980, Reinartz 1984). A shortcoming of this work is that we did not evaluate the effects of pathogens. While we did not observe any leaf pathogens, it is nonetheless possible that pathogens contribute to biotic resistance.

Reducing herbivory on bolting plants during only the second growing season (2009) significantly increased plant height (Figure 3). This increase in plant height has the potential to mediate ecological interactions with pollinators in the introduced range, which can in turn alter seed set. The flowers of tall mullein plants are more apt to be pollinated than flowers of short plants (Lortie and Aarssen 1999) and thus have higher levels of pollen deposition and rates of outcrossing (Carronero and Hamrick 2005). This has direct implications for plant performance because fruits that develop from cross-pollinated flowers produce more seed than fruits that develop from self-pollinated flowers (Donnelly et al. 1998). Thus, the reduced plant height that results from herbivory could decrease plant fitness through indirect interactions with mutualists. From an evolutionary perspective, reduced rates of outcrossing resulting from herbivory have the potential to reduce genetic diversity present in mullein populations.

Reducing herbivory for two growing seasons revealed that insect herbivory inhibits growth of mullein by reducing plant height, inflorescence length, and seed capsule density, which together lowers the number of seeds produced per plant, i.e., fitness. In our analyses, we separated survival from seed production of surviving plants. However, if we consider those that did not survive produce zero seeds by definition, then the reductions in seed set would be even larger.

This large reduction, however, begs the question of whether or not common mullein populations are seed limited or site limited (Maron and Crone 2006). It has often been assumed that plants such as mullein, which produce a long-lived seed bank, are buffered against herbivore-induced reductions in seed set (Crawley 2000; Louda and Potvin 1995). However, additive losses in seeds entering the soil over time could diminish the number of propagules available for recruitment. For example, modeling carried out by

Maron and Gardner (2000) illustrated that reducing the seed set of plants with long-lived seeds has the potential to lower population size in the future, a finding that was subsequently supported in experimental systems (e.g., Maron and Simms 2001). However, a reduction in seed set caused by insect herbivores may not limit the number of propagules enough – seed set must be reduced beyond the population restrictions caused by microsite limitation, for example as imposed by density-dependent seedling mortality (e.g., Garren and Strauss 2009). While we did not address density-dependent seedling mortality during our field experiment, we observed seedlings germinating in dense mats surrounding parental plants, suggesting it could be quite important to regulation of population size.

Interaction between patterns of herbivory and experimental treatments

We have shown that reducing herbivory on introduced mullein directly affects several plant performance metrics. Additionally, we have uncovered an interesting pattern that provides support for the plant vigor hypothesis, which states that herbivores prefer to feed on more vigorous or apparent plants (Price 1991; reviewed in Cornelissen et al. 2008). As expected, herbivory was lower on average among plants in the insecticide treatment, and herbivory was equally distributed across tall and short plants. In the ambient herbivory treatment, however, there was a positive relationship between damage level and several measures of plant performance. This positive correlation suggests that when plants are unmanipulated, herbivore feeding preferences track plant performance, as suggested by the plant vigor hypothesis, rather than driving it. The mechanisms that underlie the preference of herbivores for more vigorous plants are not well understood (Price 1991, Inbar et al. 2001, Cornelissen et al. 2008), but size-based variation in plant nutritional quality or levels of defense may be of importance (e.g., Inbar et al. 2001, Cornelissen et al. 2008). Regardless of the mechanism, treating plants with insecticide effectively altered the choice regime at our site in a manner that obscured the typical feeding preferences of chewing herbivores.

Conclusions

While introduced mullein has spread throughout North America, our data suggest that top-down regulation may help prevent the species from becoming more ecologically harmful. Our data also illustrate that even when introduced species escape from enemies and exhibit increased performance at a biogeographic scale, herbivores present in the introduced community can still impose biotic resistance at several points during the life cycle. Subsequent work with mullein and other relatively “benign” introduced species should explore how different forms of biotic resistance (e.g., via herbivory and competition) interact to shape population growth rates. More generally, for research on biological invasions to advance, it is imperative that we study not just the factors that facilitate invasion of successful invaders, but also those that inhibit population growth of less successful invaders.

Acknowledgments

This research was made possible with support from the Colorado Agricultural Experiment Station. We thank Larimer County for granting access to the research plot. Janet Hardin provided invaluable assistance with the experiment. RAH acknowledges the support of Fulbright France, and INRA CGBP. C. A. was partially funded by long-term research development project no. RVO 67985939 (from the Academy of Sciences of the Czech Republic) during the writing of this manuscript. This work benefitted from discussions at meetings of the Global Invasions Network, supported by supported by the US National Science Foundation research coordination program under grant no. 0541673.

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Appendix

Testing effects of insecticide on plant growth

We evaluated the effect of Bayer Advanced Dual Action Rose & Flower Insect Killer (ready-to-use) on *Verbascum thapsus* plant growth.

Seeds were collected from the field site in Loveland, Colorado in 2008. After one month at room temperature, they were moved to 4 degrees C for two weeks and subsequently kept in the refrigerator until ready for use.

The seedlings were germinated in plugs of germination soil mix that had been treated with fungicide on July 1, 2009. After one month, on July 31, plugs were transferred to 4.5 × 4.5 cm pots of fritted clay (Turface). Eight plants from each of 10 parent plants were grown for a total of 80 plants. The day that plants were transferred, they received a fertilizer treatment of 15-30-15 NPK. The plants were kept in the greenhouse

for one week after transplant, after which they were moved to an outdoor shade house. They were watered, weeded and fertilized as needed.

One week before the first treatment, all plants were treated with a 1% concentrated soap spray to remove any insects that might be on the plants. The plants were randomly assigned one of two treatments: insecticide or water. They were sprayed until wet but not dripping in a spray chamber and then moved to a greenhouse bench. The plants received a second treatment two weeks later. Two days before the second treatment, all plants again were treated with a 1% concentrated soap spray to remove any insects that might be on the plants. The plants were harvested two weeks after the second treatment and dried for one week at 40 degrees C to constant weight in paper bags, and then were weighed.

The effect of treatment on biomass was analyzed in JMP. The insecticide neither increased nor decreased plant biomass ($F_{1,66} = 0.20$, $P = 0.66$).

Supplementary figure

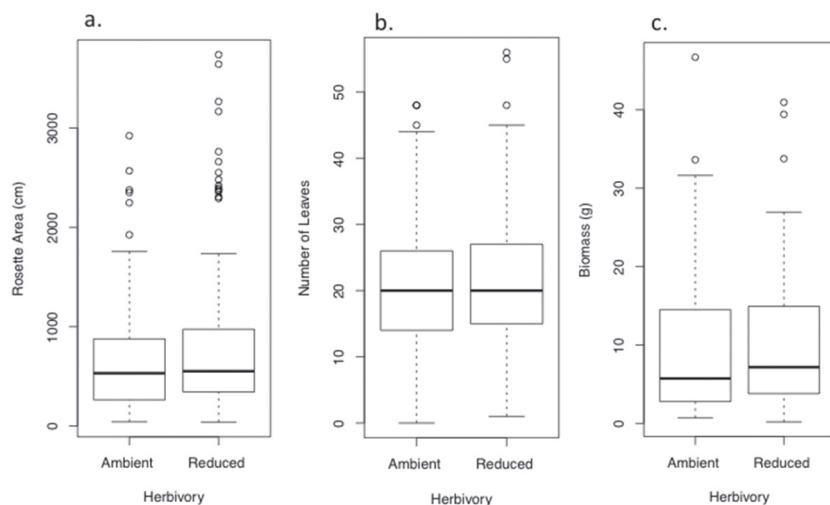


Figure S1. Box - and - whisker plots illustrating aspects of rosette size of *Verbascum thapsus* at the end of one field season of treatment (the plants' first growing season), corresponding to Figure 1. Panels show **A** final rosette area **B** number of leaves and **C** biomass.

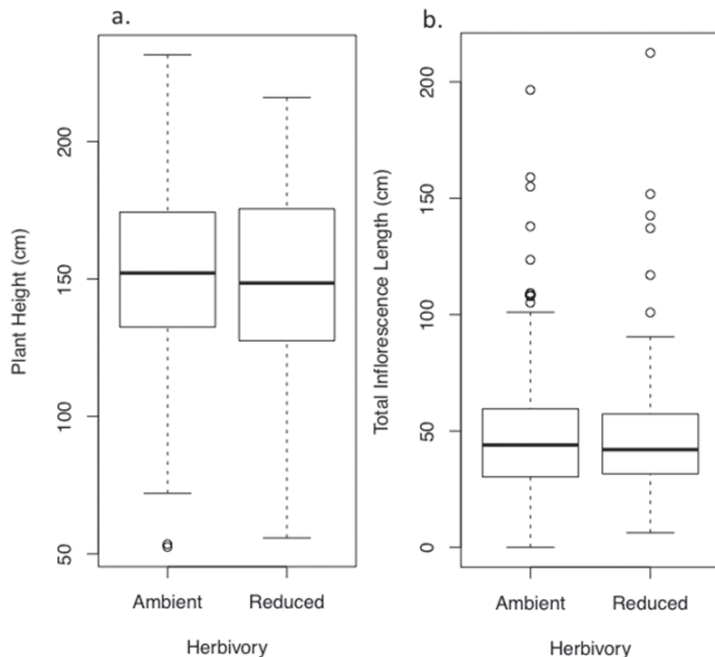


Figure S2. Box - and - whisker plots showing the characteristics of bolting plants after one field season of treatment (the plants' second growing season), corresponding to Figure 2. Panels show **A** plant height and **B** inflorescence length.

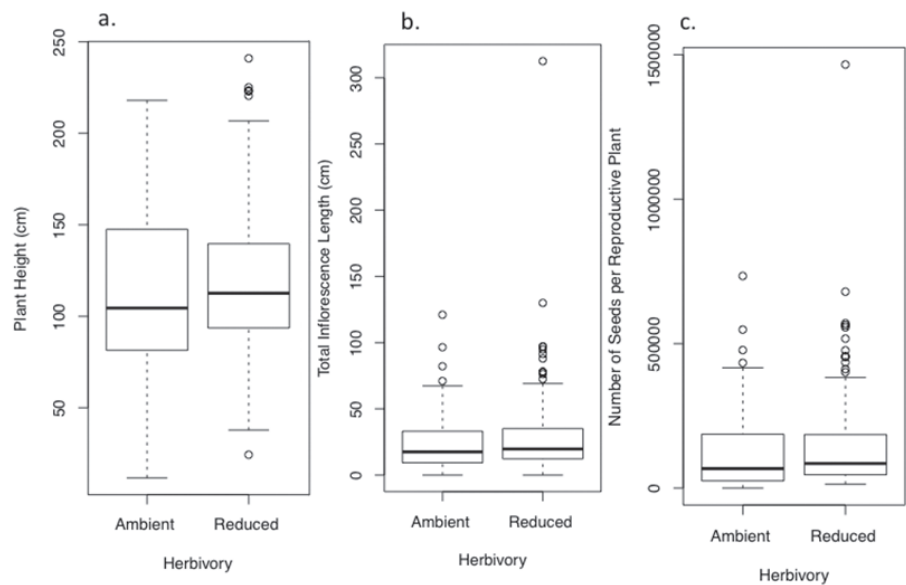


Figure S3. Box - and - whisker plots showing performance after two field seasons of treatment, corresponding to Figure 3. Panels show **A** plant height **B** inflorescence length, and **C** seed production of those plants that reproduced.

Supplemental tables

Supplemental Anova Tables. Source indicates the different factors in the analysis. DDF is denominator degrees of freedom, NDF is numerator degrees of freedom, F values and P values are also provided. See text for additional details.

Table S1a. Rosette area, 2009 (treated 1 season).

Source	DDF	NDF	F	P
Treatment	1	332.66	5.10	0.025
Cover	1	336.04	2.56	0.111
Initial Area	1	336.86	14.28	0.000
Avg. Herbivory	1	333.65	4.60	0.033
Avg. Herbivory × Treatment	1	330.66	0.00	0.998

Table S1b. Number of leaves, 2009 (treated 1 season).

Source	DDF	NDF	F	P
Treatment	1	345.40	0.78	0.377
Cover	1	344.28	0.05	0.825
Initial Area	1	348.94	23.80	<.0001
Avg. Herbivory	1	346.79	1.36	0.245
Avg. Herbivory × Treatment	1	343.18	2.91	0.089

Table S1c. Biomass of rosettes, 2009 (treated 1 season).

Source	DDF	NDF	F	P
Treatment	1	64.55	0.13	0.717
Cover	1	64.99	0.12	0.732
Initial Area	1	63.48	4.07	0.048
Avg. Herbivory	1	64.95	0.31	0.581
Avg. Herbivory × Treatment	1	63.21	0.58	0.450

Table S2a. Plant Height, Bolting plants 2009 (treated only their 2nd growing season).

Source	DDF	NDF	F	P
Treatment	1	91.13	7.03	0.009
Cover	1	85.25	0.56	0.456
Initial Area	1	77.98	58.64	<.0001
Avg. Herbivory	1	71.69	13.37	0.001
Avg. Herbivory × Treatment	1	83.59	7.29	0.008

Table S2b. Total Inflorescence Length, Bolting plants 2009 (treated only their 2nd growing season).

Source	DDF	NDF	F	P
Treatment	1	95.45	3.17	0.078
Cover	1	96.76	4.48	0.037
Initial Area	1	96.20	66.26	<.0001
Avg. Herbivory	1	96.48	10.45	0.002
Avg. Herbivory × Treatment	1	95.69	2.26	0.136

Table S3a. Plant Height, Bolting plants 2010 (treated both growing seasons).

Source	DDF	NDF	F	P
Treatment	1	235.33	10.99	0.001
Cover 2009	1	237.75	0.52	0.470
Cover 2010	1	210.71	1.78	0.184
Initial Area	1	236.72	7.23	0.008
Avg. Herbivory	1	236.26	11.20	0.001
Avg. Herbivory × Treatment	1	233.03	6.30	0.013

Table S3b. Total Inflorescence Length, Bolting plants 2010 (treated both growing seasons).

Source	DDF	NDF	F	P
Treatment	1	236.48	15.70	<.0001
Cover 2009	1	238.88	0.56	0.454
Cover 2010	1	208.58	0.80	0.373
Initial Area	1	238.38	7.12	0.008
Avg. Herbivory	1	237.27	19.13	<.0001
Avg. Herbivory × Treatment	1	233.98	1.26	0.263

Table S3c. Seed capsule density, Bolting plants 2010 (treated both growing seasons).

Source	DDF	NDF	F	P
Treatment	1	207.00	9.06	0.003
Cover 2009	1	204.78	0.05	0.822
Cover 2010	1	129.46	4.19	0.043
Initial Area	1	201.69	0.13	0.714
Avg. Herbivory	1	206.65	20.90	<.0001
Avg. Herbivory × Treatment	1	204.13	12.34	0.001

Table S3d. Total No. of Seed Capsules, Bolting plants 2010 (treated both growing seasons).

Source	DDF	NDF	F	P
Treatment	1	203.24	22.58	<.0001
Cover 2009	1	204.77	1.68	0.196
Cover 2010	1	191.22	0.08	0.777
Initial Area	1	204.86	2.74	0.099
Avg. Herbivory	1	203.53	30.09	<.0001
Avg. Herbivory × Treatment	1	200.02	9.19	0.003

The ecology, biogeography, history and future of two globally important weeds: *Cardiospermum halicacabum* Linn. and *C. grandiflorum* Sw.

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Academic editor: J. Kollmann | Received 8 April 2013 | Accepted 9 July 2013 | Published 11 October 2013

Citation: Gildenhuys E, Ellis AG, Carroll SP, Le Roux JJ (2013) The ecology, biogeography, history and future of two globally important weeds: *Cardiospermum halicacabum* Linn. and *C. grandiflorum* Sw. NeoBiota 19: 45–65. doi: 10.3897/neobiota.19.5279

Abstract

Members of the balloon vine genus, *Cardiospermum*, have been extensively moved around the globe as medicinal and horticultural species, two of which are now widespread invasive species; *C. grandiflorum* and *C. halicacabum*. A third species, *C. corindum*, may also have significant invasion potential. However, in some regions the native status of these species is not clear, hampering management. For example, in South Africa it is unknown whether *C. halicacabum* and *C. corindum* are native, and this is a major constraint to on-going biological control programmes against invasive *C. grandiflorum*. We review the geography, biology and ecology of selected members of the genus with an emphasis on the two most widespread invaders, *C. halicacabum* and *C. grandiflorum*. Specifically, we use molecular data to reconstruct a phylogeny of the group in order to shed light on the native ranges of *C. halicacabum* and *C. corindum* in southern Africa. Phylogenetic analyses indicate that southern African accessions of these species are closely related to South American taxa indicating human-mediated introduction and/or natural long distance dispersal. Then, on a global scale we use species distribution modelling to predict potential suitable climate regions where these species are currently absent. Native range data were used to test the accuracy with which bioclimatic modelling can identify the known invasive ranges of these species. Results show that *Cardiospermum* species have potential to spread further in already invaded or introduced regions in Australia, Africa and Asia, underlining the importance of resolving taxonomic uncertainties for future management efforts. Bioclimatic modelling predicts Australia to have highly favourable environmental conditions for *C. corindum* and therefore vigilance against this species should be high. Species distribution

modelling showed that native range data over fit predicted suitable ranges, and that factors other than climate influence establishment potential. This review opens the door to better understand the global biogeography of the genus *Cardiospermum*, with direct implications for management, while also highlighting gaps in current research.

Keywords

Balloon vines, biological invasion, *C. corindum*, management, phylogeny, species distribution modelling

Rationale

Understanding the biology, ecological requirements, and native distributions of potentially invasive species is crucial to ensure effective management and to predict their potential invasiveness. We review these attributes for selected members of a globally weedy genus, *Cardiospermum*, commonly known as balloon vines. We review the ecology and history of anthropogenic range expansion of the genus, with special emphasis on the two most problematic species in the group, *C. grandiflorum* and *C. halicacabum*. On a regional scale we aim to resolve the native provenance(s) of balloon vine species found in southern Africa, using a phylogenetic approach. Lastly, on a broad scale we assess the invasion risk posed by balloon vine species found outside their supposed native ranges, using species distribution modelling. Moreover, to evaluate the merit of this commonly employed method, we compare data of known invaded areas to predictions based on native range records.

Biogeography and phylogeny of selected *Cardiospermum* taxa

The genus *Cardiospermum* L. 1753 (family Sapindaceae, tribe Paullinieae) currently consists of 17 shrub, subshrub, climber, and erect species, commonly called balloon vines (Subramanyam et al. 2007). Around half of the species occur in moist tropical and subtropical regions while others are arid-adapted (Ferrucci and Urdampilleta 2011). Thirteen *Cardiospermum* species (*C. oliveirae*, *C. urvilleoides*, *C. procumbens*, *C. pterocarpum*, *C. anomalum*, *C. pygmaeum*, *C. cristobaliae*, *C. tortuosum*, *C. bahianum*, *C. integerrimum*, *C. heringeri*, *C. cuchujaquense*, *C. dissectum*) are mostly restricted in and around the Neotropics from south-eastern Brazil to north-central Mexico (Ferrucci and Umdiriri 2011) with most found in Brazil (12 spp.). Nine species are restricted to Brazil while the remaining eight species display wider geographical distributions. *Cardiospermum pterocarpum* occurs in Brazil, Argentina and Paraguay. *Cardiospermum pygmaeum*, *C. dissectum* and *C. cuchujaquense* are restricted to Mexico with *C. dissectum* also having been recorded in Texas, USA. *Cardiospermum pechuelii* is the only taxon restricted to Africa, occurring only in the desert areas of Namibia. Three species, *C. corindum*, *C. halicacabum* and *C. grandiflorum* have near cosmopolitan distributions (Ferrucci and Umdiriri 2011, Urdampilleta et al. 2012).

Morphology divides this genus into three sections; *Cardiospermum* Radlk., *Carphospermum* Radlk. and *Ceratadenia* Radlk. (Urdampilleta et al. 2012). In addition to *Cardiospermum*, Paullinieae includes five other genera, *Serjania*, *Paullinia*, *Urvillea*, *Houssayanthus* and *Lophostigma*, of which *Urvillea* is regarded the sister genus to *Cardiospermum* (Ferrucci and Acevedo-Rodriguez 1998).

Only four *Cardiospermum* species occur abundantly outside the neotropics: *C. halicacabum*, *C. grandiflorum*, *C. corindum*, and *C. pechuelii* (Burke 2003, Ferrucci and Umdiriri 2011). *Cardiospermum pechuelii* may be the only true African taxon, found in the Namib Desert (Burke 2003, Simelane et al. 2011). *Cardiospermum pechuelii* is morphologically similar to other arid adapted species, such as *C. dissectum* from Mexico. The most widely distributed species are tropical and subtropical *Cardiospermum corindum* (Fig. 1A), *C. grandiflorum* (Fig. 1B) and *C. halicacabum* (Fig. 1C) (Mc Kay et al. 2010, Simelane et al. 2011). All three species occur in the Neotropics and subtropical southern Africa. *Cardiospermum corindum* is also found in parts of India where it is known under its synonym name *C. canescens* (The Plant List 2010, Raju et al. 2011). *Cardiospermum grandiflorum* and *C. halicacabum* are present in Australia and other Pacific islands classified as alien or invasive, and *C. halicacabum* is also present in Europe and Asia (Subramanyam et al. 2007). In many of these countries the native status of these species is highly debated and their biogeographical history remains uncertain (Table 1). *Cardiospermum grandiflorum*, *C. corindum* and *C. halicacabum* are regarded as being native in South and Central America while the status of *C. halicacabum* is questioned in North America (Henry and Scott 1981, Bowen et al. 2002, Carroll 2007, Goosem 2008) and tropical Africa (USDA, United States Department of Agriculture; Weeds of Australia). Similarly the status of *C. corindum* is uncertain throughout the African continent (Henderson 2001, Simelane et al. 2011). In Asia *C. halicacabum* is variously regarded as either alien or native (Venkatesh and Krishnakumari 2006, Subramanyam et al. 2007).

Invasion history of the genus *Cardiospermum*

Alien invasive species are a global concern and a threat to biodiversity (Pimentel et al. 2000, Van Wilgen et al. 2001). They also negatively impact agricultural and forestry sectors with substantial economic costs associated with their direct impacts, eradication, control and restoration efforts (Pimentel et al. 2000, 2001). Like many invasive species, *Cardiospermum* species have been introduced for their economic value prior to becoming problematic (Pimentel et al. 2000, Van Wilgen et al. 2001). *Cardiospermum* species have been extensively moved around the world for both their medicinal (Venkatesh Babu and Krishnakumari 2006, Subramanyam et al. 2007) and ornamental (Carroll et al. 2005a) values.

The ornamental attraction of *Cardiospermum* species are their inflated balloon shaped fruit (Fig. 2). Coincidentally this trait also contributes to their colonisation success, since these balloons can float in seawater and stay viable for long periods of time, facilitating long distance dispersal, even between landmasses (Carroll et al. 2005a, Simelane et al. 2011). For example, *C. grandiflorum* was introduced to the Cook Islands as a result of a

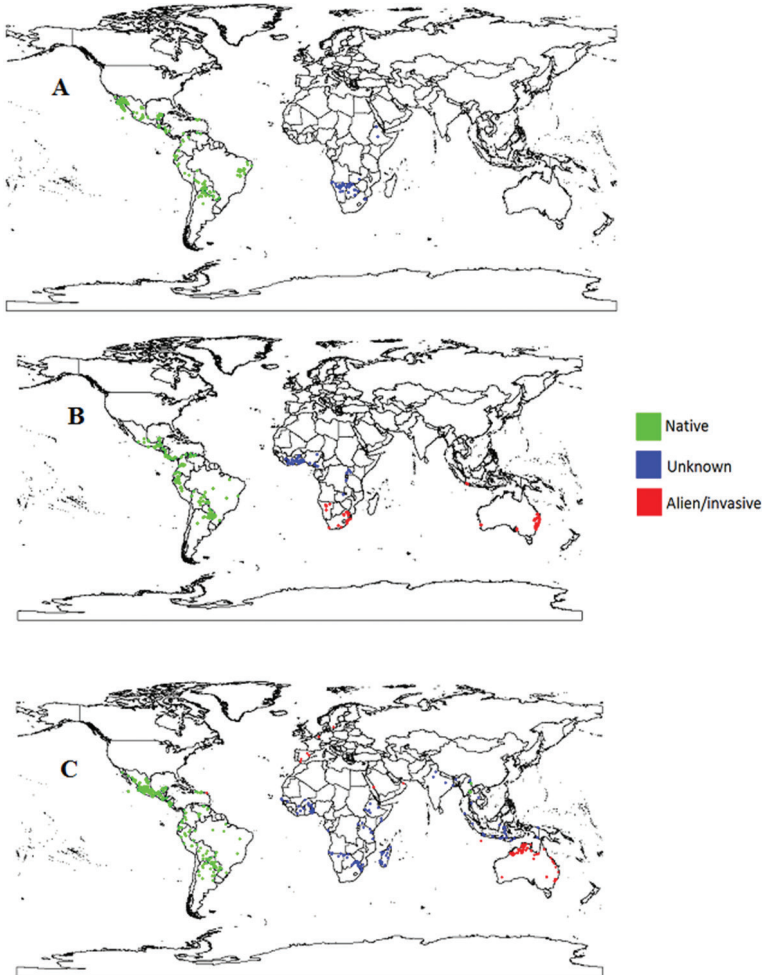


Figure 1. Distribution of *Cardiospermum* species. Global distribution of **A** *C. corindum* **B** *C. grandiflorum* and **C** *C. halicacabum* in native, unknown and alien or invasive regions.

hurricane (Meyer 2004), whilst increased spread of balloon vines in Australia was associated with a major cyclone and subsequent flooding (Carroll et al. 2005a). We floated *C. grandiflorum* fruit structures in seawater and found some of them capable of floating more than 25 weeks with seed remaining viable. (E. Gildenhuys et al., unpubl. data). Upon dehiscence, each seed is attached to a circular blade that permits further transport by wind.

Invasive *Cardiospermum* species are considered “transformer weeds” (Mc Kay et al. 2010), as they often extensively cover native vegetation, depriving it of sunlight and thus photosynthesis (Mc Kay et al. 2010, Simelane et al. 2011). *Cardiospermum* invasions also have substantial economic impacts on sugarcane and soybean production (Johnston et al. 1979, Jolley et al. 1983, Voll et al. 2004, Subramanyam et al. 2007, Murty and Venkaiah 2011). For example, in Brazil *C. halicacabum* reduces soybean crop yields by

Table 1. Details of uncertain native or non-native statuses of two *Cardiospermum* species in North America and Africa.

	Continent	References for debated native/non-native status
<i>C. halicacabum</i>	North America	Brizicky 1963, James 1825, Carroll and Boyd 1992
	Africa	Brizicky 1963, Davies and Verdcourt 1998, Hyde et al. 2012a, Hyde et al. 2012b, Henderson 2001, Foxcroft et al. 2008, Simelane et al. 2011
<i>C. corindum</i>	Africa	Davies and Verdcourt 1998, Henderson 2001, Simelane et al. 2011, Germishuizen et al. 2006, Adeyemi and Ogundipe 2012
	North America	Brizicky 1963, Castellanos et al. 1999, Molina-Freaner and Tinoco-Ojanguren 1997

up to 26% (Dempsey et al. 2011, Brighenti et al. 2003). The problem with controlling *Cardiospermum* infestations in soybean crops is the difficulty of mechanically excluding their seeds, which are similar in size and shape to those of soy (Brighenti et al. 2003).

Two balloon vine species well-travelled

Currently two *Cardiospermum* species are globally considered important invaders. *Cardiospermum grandiflorum* is classified as an invasive species in Australia, southern Africa, Cook Islands and many other Pacific islands (Mc Kay et al. 2010) while *C. halicacabum* is considered a weed in Australia with its status (native or introduced) undetermined in most other parts of its range (Henderson 2001, Harris et al. 2007). In Australia, *C. grandiflorum* is considered amongst the “most destructive life forms of rainforests” (Werren 2002), while in South Africa *C. grandiflorum* is classified as a Category 1 weed which means its cultivation is prohibited and control is mandatory (Henderson 2001).

South Africa’s Working for Water program launched a research initiative in 2003 to find biological control agents against *C. grandiflorum* (Simelane et al. 2011). Eight insects and two fungal agents have been identified and are currently undergoing host-specificity testing in South Africa (Simelane et al. 2011). Most are capable of feeding and developing on other *Cardiospermum* spp. in South Africa, in particular *C. halicacabum* and *C. corindum* (Mc Kay et al. 2010). Three promising agents were identified, a seed-feeding weevil (Curculionidae: *Cissoanthonomus tuberculipennis*), a fruit-galling midge (Cecidomyiidae: *Contarinia* spp.) and the rust fungus *Puccinia arechavaletae* (Simelane et al. 2011). Concerns about potential non-target impacts of candidate control agents on *C. corindum* and *C. halicacabum*, as well as the debated native status of these congeners in southern Africa (Table 1), have so far prevented the release of these agents.

Invasion histories of *C. grandiflorum* and *C. halicacabum*

The ornamental trade of *Cardiospermum halicacabum* and *C. grandiflorum* spans more than 100 years. For example, in Australia the first herbarium records of *C.*

grandiflorum date back to 1923, collected around Sydney, New South Wales (Carroll et al. 2005a). Currently invasive populations are found throughout the east coast of Australia between Sydney and Cairns although less abundantly to the north of Brisbane (E. Gildenhuys, pers. obs.). More recently the species has spread inland to forest areas such as Toowoomba (Queensland) and the Blue Mountains (New South Wales) (Carroll et al. 2005a, E. Gildenhuys, pers. obs.). *Cardiospermum halicacabum* is more abundant in the northern parts of Australia such as Darwin and Cairns, and is seldom found along the east coast south of Rockhampton, Queensland (E. Gildenhuys, pers. obs.). It is speculated that *C. halicacabum* was introduced during James Cook's second voyage in the 1770's long before the introduction of *C. grandiflorum* (Bean 2007, Harris et al. 2007).

The introduction of *Cardiospermum grandiflorum* into South Africa occurred approximately 100 years ago (Simelane et al. 2011). Today it is classified as a major weed, and is present and considered invasive in five provinces, of which Kwazulu-Natal and the Eastern Cape are the most affected (Henderson 2001, Simelane et al. 2011). The first records of *C. halicacabum* in South Africa dates back to 1917, 1919 in Namibia and 1930 in Botswana (Global Biodiversity Information Facility: GBIF, <http://data.gbif.org/welcome.htm>). It is classified as a minor weed in southern Africa, though its native status is debated, with slight impacts compared to *C. grandiflorum* (Henderson 2001).

Cardiospermum halicacabum and *C. grandiflorum* are also present in North America (Carroll and Loye 2012). *Cardiospermum halicacabum* is more widespread than *C. grandiflorum*, the latter apparently restricted to a small area in suburban Los Angeles (S. Carroll, pers. obs.). Due to the evident ability of some *Cardiospermum* species to disperse over long distances (Carroll et al. 2005a, Simelane et al. 2011), it is possible that the presence of *C. halicacabum* in North America is due to natural dispersal from South and Central America, rendering a native status. On the other hand, if seeds escaped horticultural and agricultural environments, they should be awarded non-native status (Subramanyam et al. 2007). *Cardiospermum halicacabum* was reported in the Spontaneous Illinois Vascular Flora before 1922 and was described as abundant in Oklahoma in the 1820's (James 1825); thus, if not native, *C. halicacabum* was introduced more than 180 years ago.

Cardiospermum halicacabum is also present in China and India. In China it is described as a common weed in forest margins, shrublands, grasslands, cultivated areas and wastelands of the east, south and southwest (Flora of China, www.eFloras.org) – though considered native by some – [Pacific Island Ecosystems at Risk (PIER)]. In India it is widespread and considered non-native (Raju et al. 2011). The history of *C. halicacabum* in these countries is unknown, but it is widely used for medicinal purposes (Subramanyam et al. 2007).

Biology and ecology of *C. grandiflorum* and *C. halicacabum*

A comprehensive understanding of the biology and ecology of *C. halicacabum* and *C. grandiflorum* is important because of the invasive potential and biogeographic uncer-

tainties which characterise these two taxa. Such information will also contribute to making informed decisions on their conservation (if native) or control (if invasive). This is especially true since the extent to which these species are invasive is essentially unknown and the uncertainties of their classification in most areas suggest the possibility of a cosmopolitan native distribution.

The morphology of these two species is similar, with both being adapted for tropical and subtropical climates. *Cardiospermum grandiflorum* is a large, semi-woody perennial, whereas *C. halicacabum* is smaller, less woody and commonly annual. *Cardiospermum grandiflorum* has elongated fruit (4.5–6.5 cm in length) compared to the more compact fruit of *C. halicacabum* (2.5–3.0 cm in length) (Fig 2A and B). Fruit structures consist of three dorsally keeled membranous capsules each consisting of three internal blades (Weckerle and Rutishauser 2005). The fruit are septifragal with the capsules breaking away from each other when fruit are ripe, changing colour from green to brown (Weckerle and Rutishauser 2005). Seeds of the two species differ, with a kidney shaped hilum on *C. halicacabum* seeds and a round hilum on *C. grandiflorum* seeds. Both species normally produce three seeds per fruit (Weckerle and Rutishauser 2005), are climbers with tendrils and have large flat biternate leaves. The leaves and stems of *C. grandiflorum* have small reddish hairs that are absent in *C. halicacabum* (Henderson 2001). Flowers are white and yellow with *C. halicacabum* flowers smaller (2–3 mm) compared to those of *C. grandiflorum* (7–11 mm) (Henderson 2001). The average length of *C. halicacabum* is 1–3 m, while *C. grandiflorum* is slightly taller with an average of 2–5 m, though both are capable of greatly exceeding these lengths (Henderson 2001).

Both taxa produce flavone aglycones and cyanogenic compounds that likely protect them against predators such as soapberry bugs (Subramanyam et al. 2007). Soapberry bugs (genera *Leptocoris*, *Jadera* and *Boisea* from the family Rhopalidae) feed exclusively on seeds of Sapindaceae and are predators of *Cardiospermum* (Carroll et al. 2005b, Carroll 2007). An example of the impact of invasive *Cardiospermum* populations includes an evolved increase in beak length of the native *Leptocoris tagalicus* soapberry bug feeding on invasive *C. grandiflorum* in Australia (Carroll et al. 2005b). Soapberry bugs co-occur with the widespread distribution of *Cardiospermum* and thus may be a factor in *Cardiospermum* reproduction globally. A treatment of soapberry bugs that feed on *C. halicacabum* and *C. grandiflorum* can be found in Carroll and Loye (2012).

The germination and growth success of *Cardiospermum halicacabum* is well studied because of its medicinal value, as well as its impact on soybean plantations and on natural riparian areas (Dempsey 2011). In contrast, no studies exist addressing these topics for *C. grandiflorum*, despite the need for additional biological information about this environmental weed. Optimum germination of *C. halicacabum* takes place at 35°C, with high oxygen concentrations increasing germination success (Johnston et al. 1979, Jolley et al. 1983, Dempsey 2011). Therefore, in natural habitats, establishment may be more likely in conditions with warm, well-oxygenated soils. Seeds and young plants are able to survive flooded, saturated and dry conditions while performing best in intermediate conditions (Dempsey 2011).

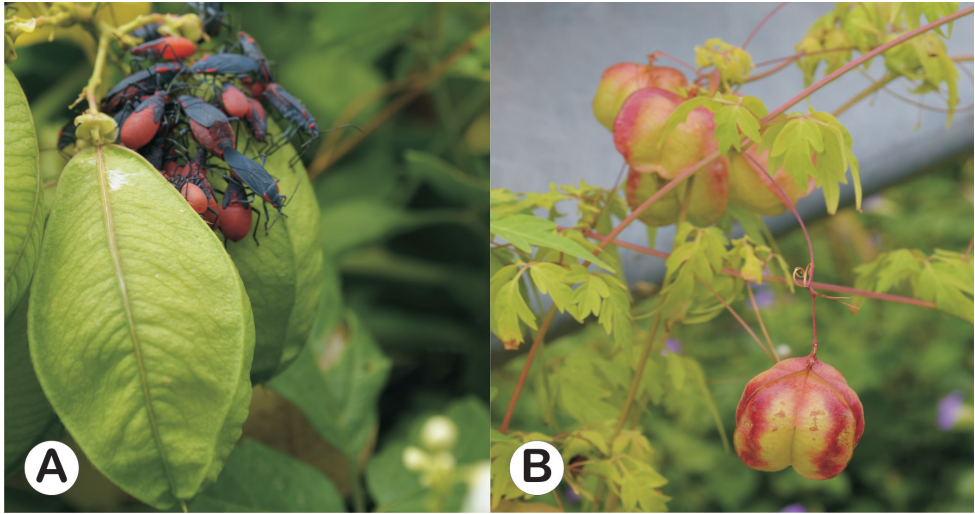


Figure 2. *Cardiospermum* fruit. The ornamental attraction of *Cardiospermum* plants and the reason for their widespread distribution is their balloon shaped fruit **A** *C. grandiflorum* (JJ Le Roux) and **B** *C. halicacabum* (JJ Le Roux).

Despite morphological similarity, these two species differ markedly. They occasionally occur sympatrically but mostly prefer different habitats with *C. halicacabum* dominating tropical and *C. grandiflorum* subtropical areas (Henderson 2001). Although both species invade forest margins and watercourses, *C. grandiflorum* also thrives in disturbed urban open areas while *C. halicacabum* predominantly invades wood- and grasslands which highlights its threat to plantations (Henderson 2001).

Management of invasive *Cardiospermum*

To date, managing and reducing impacts of *Cardiospermum* invasions has mostly involved manual removal or burning (Subramanyam et al. 2007). Manual removal involves cutting plants at the base enabling the top part to die off after which roots are dug out which is thus labour intensive (Mc Kay et al. 2010). Chemical control of larger plants includes treatment with paraquat, glufosinate-ammonium, lactofen, carfentrazone-ethyl, sulfentrazone, glyphosate or 2, 4-dichlorophenoxy acetic acid (Subramanyam et al. 2007). However, the use of chemical control could potentially be problematic for two reasons, firstly because of non-target impact on underlying vegetation and secondly the typical proximity of invasions to waterways makes environmental contamination a threat (Simelane et al. 2011). Another key problem in the management of *Cardiospermum* invasions is the persistent seed bank. If the weedy canopy is cleared it opens the door for long-lived seeds to sprout (FloraBase 2012).

Management and problems in South Africa

In collaboration with South Africa's Working for Water program, a biological control programme was initiated against *C. grandiflorum* in 2003. However due to the taxonomic uncertainty surrounding *C. halicacabum* and *C. corindum* (discussed earlier, Table 1), biocontrol agents cannot be released, hampering effective management in South Africa. The importance of clarifying the geographic native ranges of all *Cardiospermum* species currently found in South Africa for the successful biological control of *C. grandiflorum* is therefore evident. If *C. corindum* and *C. halicacabum* are indeed native to southern Africa, only agents that are specific on *C. grandiflorum* can qualify for release in South Africa, and thus far, these agents have proved particularly difficult to rear and test under quarantine conditions (D. Simelane, pers. comm.). On the other hand, if *C. corindum* and *C. halicacabum* are not native to southern Africa, all suitable agents against *C. grandiflorum* qualify for release in South Africa.

Molecular systematics of *Cardiospermum* species in southern Africa

To determine the relationship between *Cardiospermum* species occurring in Africa and South America we sequenced two accessions of *C. grandiflorum*, *C. halicacabum* and *C. corindum* from each continent (South America and Africa). DNA was extracted from dried plant material using the CTAB method (Doyle and Doyle 1990). The internal transcribed spacer gene region was amplified using primers ITS1 and ITS4. A phylogenetic tree was then reconstructed in BEAST version 17.4 (Drummond et al. 2012) using a General Time-Reversible (GTR + G) model with uneven rates of evolution between base pairs.

The retrieved phylogeny indicates a close relationship between samples from South America and southern Africa (Fig. 3). For *C. grandiflorum* and *C. halicacabum* southern African samples are more closely related to South American samples than to other samples from southern Africa (i.e. geographic paraphyly). It is therefore likely that *C. halicacabum* in southern Africa, like *C. grandiflorum*, represents a recent introduction, and is therefore not native. For *C. corindum* however the phylogeny cannot dismiss natural long distance dispersal as an explanation for the species' presence in southern Africa, due to the southern African accessions forming a monophyletic group within the South American clade. The ability of *Cardiospermum* fruit to float in seawater for long periods of time and remain viable, makes a strong case for long distance dispersal. In order to clarify the uncertainty around human introduction versus rare long distance dispersal events, future phylogenetic analyses should include more and geographically widespread collections.

Bioclimatic preferences of *Cardiospermum halicacabum*, *C. grandiflorum* and *C. corindum*

Prevention is better than cure, with eradication of introduced species typically becoming less feasible as spread progresses (Thuiller et al. 2005). Identifying a species' suitable

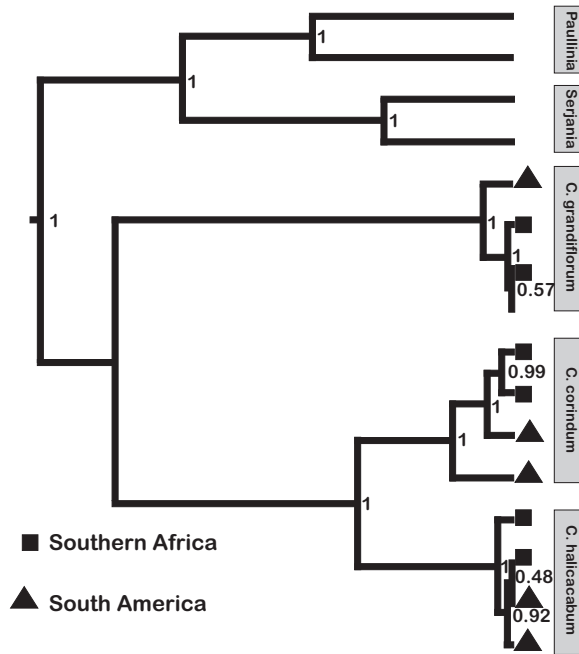


Figure 3. *Cardiospermum* phylogeny. Phylogeny of six South American and six southern African accessions of *Cardiospermum* species with *Paullinia* and *Serjania* species used as outgroup taxa. Topology support is shown as posterior probability at each node.

climatic range can therefore help to determine areas where introduction should be prevented or management intensified. Species distribution modelling is probably the most popular method for determining such areas (Allouche et al. 2006, Hirzel et al. 2006). Essential to the accuracy of species distribution modelling is the assumption that niche shifts do not occur in a newly introduced area, which has been shown to occur rarely (Petitpierre et al. 2012).

Modelling methods

We used BIOMOD version 1.1.5 (Thuiller et al. 2009) implemented in R version 2.15.1 (R Development Core Team 2012) to predict potentially suitable climate habitats for *C. halicacabum*, *C. grandiflorum* and *C. corindum*. Locality records were sourced from public databases [GBIF; Henderson 2007] and personal observations. We discarded records with spatial uncertainty (e.g., points in the ocean) and those from botanical gardens or with missing or duplicate values. Since no absence data is available for *Cardiospermum* species, but is needed for modelling, 10,000 pseudo-absence background points were created per species, by random sampling of the Köppen-Geiger climate classification. We employed generalized boosted regression models (GBM), a method uniting regression trees with boosting (for a more comprehensive

Table 2. Contribution (%) of each BioClim variable used for distribution modelling of *Cardiospermum* species. The first value in each species column is for global and the second for native range modelling.

Variables used for modelling	Variable importance					
	<i>C. halicacabum</i>		<i>C. grandiflorum</i>		<i>C. corindum</i>	
	Global	Native	Global	Native	Global	Native
Min temperature of the coldest month	21.2	12.5	13.8	25.4	14.9	21.1
Max temperature of the warmest month	6.2	2.3	4	0.9	3.9	1.7
Precipitation of the coldest quarter	4.9	22.2	27.8	25.9	13.7	2.1
Precipitation of the driest month	2	1.1	13.1	2.5	3.6	16.9
Precipitation of the warmest quarter	44.5	8.3	20	22.1	31.5	2.7
Temperature seasonality	17.2	57.6	22.8	24.9	6	7.9
Precipitation of the wettest quarter	-	-	3.2	5.7	34	42.5

description see Elith et al. 2008). For all analyses, seven climatic variables were sourced from BioClim (Hijmans et al. 2005), based on their importance for species survival and low co-linearity (Table 2). Importance, and thus the contribution of each variable to the model was assessed using Pearson rank correlation between standard predictions and those based on random permutations for each variable separately (Thuiller et al. 2009). If correlations between these two predictions were high, the specific variable was regarded as less important. Co-linearity between different variables was limited to <0.70 using Spearman rank correlation coefficients. Consequently, precipitation of the wettest quarter was dropped for modelling of *C. halicacabum* due to a high correlation with precipitation of the warmest quarter. A raster of 6 arc min was used to extract variables since a more coarse resolution is realistic for global scale prediction, while also accounting for sampling error. Models were calibrated with 70% of the data and evaluated with the remaining 30%. A cut-off value was determined with BIOMOD's default setting, representing the best probability threshold which maximizes the percentage of presence and absences correctly predicted for the evaluation data (Thuiller et al. 2009). Area under the receiver-operator-curve (AUC, Hanley and McNeil 1982) and the true skill statistic (TSS, Allouche et al. 2006) were used for model evaluation. AUC scores between 0.95 and 1 indicate an excellent, 0.9 and 0.95 a good and 0.6 and 0.8 a fair model (Thuiller et al. 2005). TSS values of 0.8–1 are excellent, 0.6–0.8 good and 0.0–0.6 fair for predicting accuracy (Allouche et al. 2006).

The accuracy of species distribution modelling is influenced by false positives and negatives (Thuiller et al. 2005, Fawcett 2006). Therefore a second aim of our species distribution modelling approach was to evaluate the accuracy with which this technique can predict potential invasive regions using models calibrated with native range data only. South and Central America were used as the native range for all three species since native status is debated in all other regions. A model calibrated using these records were then used to project suitable climate regions globally as described above. Known global occurrence records were then used as independent data to evaluate modelling accuracy.

Modelling results

Australia: Global data models for all three species performed well, with AUC values above 0.9 and TSS values above 0.65 (Table 3). Bioclimatic predictions show that a large proportion of Australia is climatically suitable for *Cardiospermum corindum*, a species currently absent in this country. Both *C. halicacabum* and *C. grandiflorum* have been introduced to Australia and are classified as invasive weeds. The suitable climate range for *C. corindum* in Australia is much larger than predicted for both *C. grandiflorum* and *C. halicacabum* and as such ornamental or medicinal introductions of *C. corindum* into Australia should be prevented (Fig. 4A, B, C). Modelling also predicted that the east coast of Australia is climatically highly suitable for *C. halicacabum*, such that any risks from its establishment in this area should be assessed. *Cardiospermum grandiflorum* appears to be a more rapid colonizer than *C. halicacabum* in Australia and it is already present in most predicted areas. It is however likely to become locally more abundant in areas where it is already found (Fig. 1B and Fig. 4B).

Europe and Asia: Our modelling approach identified Europe as mostly climatically unsuitable for *Cardiospermum* (Fig. 4A, B, C). Areas of suitable climate are present for all three species in certain parts of Asia including India (where *C. halicacabum* and *C. corindum* are present), Thailand and Pakistan, with *C. grandiflorum* potentially being the most restricted taxon (Fig. 4B). *Cardiospermum corindum* has high climatic suitability in southern Yemen, southern India, Thailand, Myanmar and southern China (Fig. 4A). The southernmost tip of Yemen seems climatically suitable for *C. halicacabum*, with India, Thailand, Cambodia, Vietnam, Myanmar, Japan, Taiwan and parts of China highly suitable (Fig. 4C). Many of these regions are already occupied by *C. halicacabum*. Climatically suitable habitat for *Cardiospermum grandiflorum* in Asia only appears to be present in southern India, Sri Lanka and parts of Vietnam (Fig. 4B).

Southern Africa: In South Africa bioclimatically suitable areas for *C. grandiflorum* are in the Western Cape Province, while for *C. halicacabum* they are in coastal areas in the Eastern Cape Province. Bioclimatically suitable areas in South Africa are the largest for *C. corindum*, with the Western and Eastern Cape Provinces being highly suitable. Currently the species is limited to Limpopo, Mpumalanga and northern parts of Kwa-zulu Natal (SANBI). Spread and anthropogenic movements of *Cardiospermum* species

Table 3. Evaluation of modelling predictions. True skill statistic (TSS) and area under the receiver operating characteristic (ROC curve) (AUC) for global and native range modelling of three widespread *Cardiospermum* species. The first value in TSS and AUC column is for global and the second for native range modelling. Independent data evaluation is for the native range models evaluated against known non-native ranges.

Species	TSS		Independent data (TSS)	AUC		Independent data (AUC)
	Global	Native		Global	Native	
<i>C. halicacabum</i>	0.651	0.703	0.441	0.9	0.923	0.755
<i>C. grandiflorum</i>	0.759	0.665	0.343	0.95	0.895	0.639
<i>C. corindum</i>	0.689	0.629	0.565	0.905	0.896	0.881

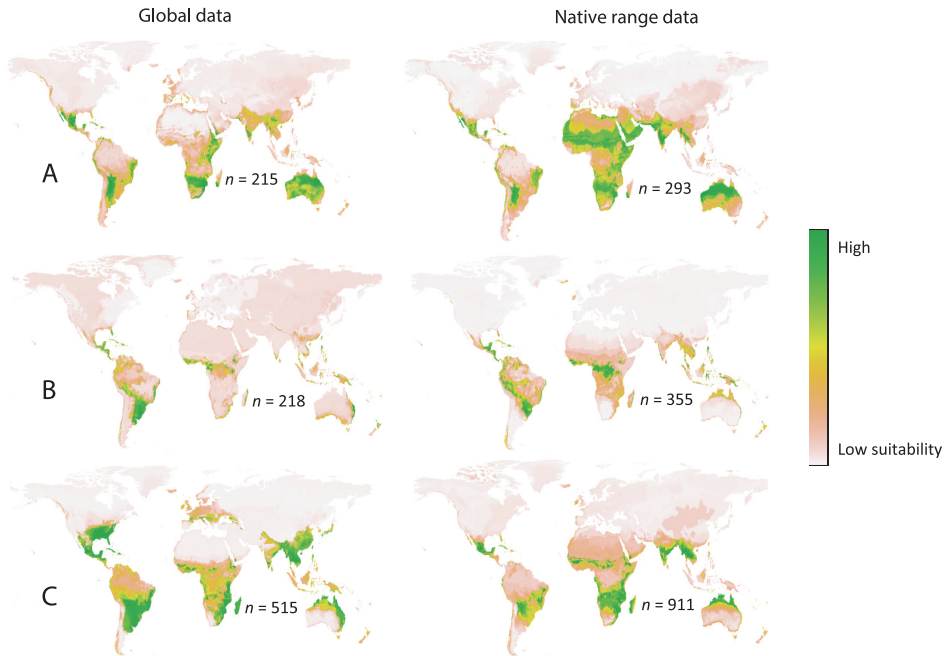


Figure 4. Species distribution modelling of *Cardiospermum* species. Global climatically suitable ranges for **A** *C. corindum* **B** *C. grandiflorum*, and **C** *C. halicacabum* as predicted by boosted regression trees in BIOMOD using global (left) and native range data (right). Number of occurrence points used for modelling (n) is indicated on each map.

in South Africa should therefore be closely monitored since a large part of South Africa appears climatically suitable for establishment. While *Cardiospermum grandiflorum* and *C. halicacabum* are recorded as naturalised in parts of Namibia and Botswana, bioclimatic modelling did not predict either country as climatically suitable. *Cardiospermum* species are not widespread in these two countries and possibly only occur in areas with suitable microclimates. Such habitats typically differ significantly from surrounding environments and often result from human actions, and are therefore excluded in bioclimatic modelling based on more coarse data, such as this study (Kearney and Porter 2009).

Testing model accuracy

Models calibrated with South and Central American native occurrence records performed fairly well when cross-validated using AUC and TSS, with values higher than 0.85 and 0.6 respectively. However this was not the case when these models were evaluated with independent data, thus known presence data not used in modelling. *Cardiospermum halicacabum* and *C. grandiflorum* had low AUC and TSS values ranging between 0.60–0.80 and 0.30–0.45 respectively, only *C. corindum* models performed fairly well (AUC > 0.85 and TSS > 0.55, Table 3).

These results indicate that models calibrated with native range occurrence records only, would not have accurately predicted the invasive spread of *C. grandiflorum* in South Africa while underestimating its potential range in Australia. This lack of accuracy for identifying invasive regions using native data questions the suitability of using species distribution modelling alone when determining potential invasive regions.

Also contrary to what we expected, models calibrated using native range data predicted larger climatically suitable areas than models calibrated with global range data (Fig. 4; except for *C. halicacabum*). We hypothesised that this is due to the more restricted climate zones created with the widespread pseudo-absence data of the global range, thus including more diverse habitats to exclude as suitable areas. We plotted the presence and absence points for both native and global range data for each variable against the probability of occurrence using the response plot function in R (Appendix, Fig. S1 A–F). In these figures it is clear that global data variables include a wider environmental range for pseudo-absences compared to the native range pseudo-absences, especially when considering the most significant variables based on variable importance (Table 2). To test if this is indeed the case we ran three additional models with the same settings as the previous models but using native range presence data and global pseudo-absences data. We used the same evaluation parameters as for the previous models (Appendix, Table S1, S2). This approach resulted in projections that more closely resembled global range model predictions or are even more restricted predictions (Appendix, Fig. S2). These results indicate that while native range data can be used to predict potential suitable areas, data are often over-fitted, thus over predict the extent of suitable habitats, due to less restricted absence data created from the native range.

Usefulness of bioclimatic species modelling

While species distribution modelling is a popular tool for predicting potential invasive ranges its accuracy remains questionable (Araújo and Luoto 2007, Sinclair et al. 2010). Bioclimatic modelling did not accurately predict current invasive regions for the widely naturalized species *C. grandiflorum*. Also native range data alone led to an over estimation of potential suitable habitats for *C. corindum* and *C. grandiflorum*. Our results comparing predictions based on native and global occurrence records are surprising and significant. We hypothesized that the reason for this observation is the more restricted climate zones created when using global pseudo-absences for model calibrations, an effect that can potentially be amplified for species characterised by incomplete range filling in their native ranges. A key assumption of species distribution modelling is pseudo-equilibrium, however this is probably unrealistic for most species and may therefore seriously impact model accuracy (Guisan and Thuiller 2005). On the other hand, bioclimatic predictions may be hampered if a species has undergone a niche shift in its invasive range (Broennimann et al. 2007). All the above-mentioned issues highlight how factors other than climate may play a crucial role in the accuracy of species distributions modelling. For example niche shift in the non-native range could be the result of release from natural enemies

(Keane and Crawley 2002). Similarly, increased resource availability in the introduced range (Davis et al. 2000, Thompson et al. 2004) may increase habitat suitability while abiotic attributes of the new range may permit spread into novel habitats. In concert, dispersal limitations (Pulliam 2000), anthropogenic effects and unique historical factors (Jiménez-Valverde et al. 2008) may limit the distribution of species in their native ranges.

Thus, taking the contradicting results into account and also considering the many other factors that influence a species distributional range, lead us to conclude that while bioclimatic modelling is a useful approach, it should not be used as a stand-alone tool when making conservation decisions regarding the introduction of species into a novel range and caution should be exercised to ensure the quality of input data while also taking other factors into account as discussed above.

Conclusions

Many regions globally appear climatically suitable for establishment of *Cardiospermum grandiflorum*, *C. corindum* and *C. halicacabum*, cautioning against further introductions. Resolving the native ranges for these species globally is therefore important for biodiversity conservation and invasive species management. For example, our preliminary results indicate that *C. halicacabum* from southern Africa have a close relationship with South American samples, but that rare long distance dispersal cannot be ruled out as an explanation, while the split between South American and southern African *C. corindum* hints towards a native status on both continents. Future work should include a more comprehensive phylogeny to substantiate our findings, including balloon vine specimens from other biogeographic regions where the native status is known. If it is found that they are indeed alien to Africa and Asia, a risk assessment challenge lies ahead since large areas of these continents appear climatically suitable for their establishment. No *Cardiospermum* species are regarded as native in Australia, and measures to limit the spread of *C. halicacabum* and *C. grandiflorum* may be augmented with biological control measures that include native soapberry bugs that are evolving to use them more efficiently (Carroll et al. 2005b). In addition, the introduction of *C. corindum* should be prohibited based on the wide environmental suitability identified for this species in Australia.

Cardiospermum species are also used by many people in rural areas for medicinal purposes, further emphasizing a need to resolve the natal biogeographic distribution of this globally important genus to ensure its effective management, control or conservation.

Acknowledgments

We thank Dr Ingolf Kühn and the two anonymous reviewers for their constructive comments on previous drafts of the manuscript. Financial support was provided by the DST-NRF Centre of Excellence for Invasion Biology and the Working for Water Programme through their collaborative project on “Research for Integrated Management of Invasive

Alien Species”. E Gildenhuys acknowledges the South African National Research Foundation’s (NRF) Scarce Skills scholarship programme. J Le Roux also acknowledges Stellenbosch University’s Sub-committee B “Young Researchers Fund” and the NRF Thuthuka Programme for research funding. S Carroll acknowledges support from the School of Life Sciences at the University of Queensland, St. Lucia. We are grateful to Jason Donaldson and Vernon Visser for their help and advice with species distribution modelling.

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Appendix

Supporting information for species distribution modelling of *Cardiospermum* species using native range presences and global pseudo absences. (doi: 10.3897/neobiota.19.5279.app) File format: Microsoft Word Document (doc).

Explanation note: The file contains the response plots for variables used in species distribution modelling. Modelling predictions and the importance of individual variables in those models using native range presence and global absence data are also given.

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Citation: Gildenhuis E, Ellis AG, Carroll SP, Le Roux JJ (2013) The ecology, biogeography, history and future of two globally important weeds: *Cardiospermum halicacabum* Linn. and *C. grandiflorum* Sw. NeoBiota 19: 45–65. doi: 10.3897/neobiota.19.5279 The ecology, biogeography, history and future of two globally important weeds: *Cardiospermum halicacabum* Linn. and *C. grandiflorum* Sw. NeoBiota 19: 45–65. doi: 10.3897/neobiota.19.5279.app

A perceived gap between invasive species research and stakeholder priorities

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Academic editor: Ingolf Kühn | Received 21 February 2013 | Accepted 24 August 2013 | Published 11 October 2013

Citation: Bayliss HR, Stewart GB, Wilcox A, Randall NP (2013) A perceived gap between invasive species research and stakeholder priorities. NeoBiota 19: 67–82. doi: 10.3897/neobiota.19.4897

Abstract

Information from research has an important role to play in shaping policy and management responses to biological invasions but concern has been raised that research focuses more on furthering knowledge than on delivering practical solutions. We collated 449 priority areas for science and management from 160 stakeholders including practitioners, researchers and policy makers or advisors working with invasive species, and then compared them to the topics of 789 papers published in eight journals over the same time period (2009–2010). Whilst research papers addressed most of the priority areas identified by stakeholders, there was a difference in geographic and biological scales between the two, with individual studies addressing multiple priority areas but focusing on specific species and locations. We hypothesise that this difference in focal scales, combined with a lack of literature relating directly to management, contributes to the perception that invasive species research is not sufficiently geared towards delivering practical solutions. By emphasising the practical applications of applied research, and ensuring that pure research is translated or synthesised so that the implications are better understood, both the management of invasive species and the theoretical science of invasion biology can be enhanced.

Keywords

Alien species, biological invasions, knowledge transfer, research evaluation, science policy

Introduction

Access to scientific information is important in ensuring an effective response to biological invasions (Browne et al. 2009, Simpson et al. 2009). This scientific information needs to be used judiciously alongside legislative and socio-economic information and local knowledge to inform decision-making (Segan et al. 2011). The scientific information generated through research activities can be broadly described as relating to the processes, impacts or management of invasive species (Kueffer and Hirsch-Hadom 2008). This information is continuously being published in both books and journals (Simberloff 2004, Richardson and Pyšek 2008), with the number of journal articles relating to biological invasions rising exponentially (Kühn et al. 2011). Whilst many journals in the fields of ecology and conservation biology publish papers on invasive species, the recent growth in the number of journals focusing specifically on biological invasions reflects the increasing demand in this area (e.g. Aquatic Invasions, Biological Invasions, Management of Biological Invasions, NeoBiota). Yet, despite the accumulation of a massive body of literature, there are still gaps in our fundamental knowledge about many invasive species (McGeoch et al. 2010). There is a need for much basic data on invasive species drawn from empirical research, which could be used to underpin theoretic ecological approaches, for example risk assessment (Andersen et al. 2004). Topics still to be explored fully relate to the underlying mechanisms, consequences, assessment, management, ecology and economics of biological invasions (Kühn et al. 2011, Williamson et al. 2011).

The IUCN Red List database implicates invasive species in the extinction of more than half of the 170 species for which data are available (Blackburn et al. 2010). To halt or reduce future global biodiversity loss, increased investment in the management of invasive species will be required (McGeoch et al. 2010). However, criticism has been made that much of the scientific literature on invasive species focuses on furthering knowledge and quantifying impacts rather than on delivering practical solutions (Hulme 2006, Esler et al. 2010). To explore this, we collated key priorities for invasive species science and management from stakeholders working with invasive species and then compared them to the topics of articles published over the same period in eight journals to determine whether the topics identified as important by stakeholders were being addressed by research, and to identify any areas that may require a greater focus in the future.

Methods

We gathered priorities for science and management from members of the international invasive species community using a combination of methods to increase participation. Hard-copy questionnaires designed to assess information use by invasive species stakeholders were distributed at two events in Great Britain; the GB Non-Native Species Secretariat Stakeholder Forum 2009 and the British Ecological Society Invasive Species Group Conference 2009. Questionnaires were anonymous, but respondents

were asked to identify their main area of responsibility (i.e. research, policy, practice, others). The questionnaires finished with a question asking respondents to identify their three top priorities for invasive species science and management. The same question was distributed to delegates attending a dedicated workshop held at the European Congress of Conservation Biology in Prague, 2009. In 2010, the question was included in an anonymous electronic questionnaire exploring information selection and sharing that was distributed to the international invasive species community using the Aliens-L email list of the Invasive Species Specialist Group of the IUCN Species Survival Commission and subsequently reposted onto other web pages and email lists by recipients. Responses from the questionnaires and the workshop were entered into a spread sheet for thematic analysis, whereby related priorities were grouped using an iterative process (see online Appendix I: Stakeholder priorities for the data used in the analyses). Priorities were also analysed by comparing responses between stakeholder groups, with the eight most frequently identified priorities (those identified a total of twenty or more times) charted to allow comparison by stakeholder group.

We then undertook a search of eight journals likely to publish research of broad relevance to invasion biology. Four journals were 'traditional' ecological journals; Biological Invasions; Diversity and Distributions; the Journal of Applied Ecology; and Trends in Ecology and Evolution. The other four were subsequently selected to broaden the scope of the study, and included Ecological Economics, Journal of Environmental Management, Weed Research and Conservation Evidence. Other relevant journals which did not cover the time period of 2009–2010 (such as Management of Biological Invasions or NeoBiota, which produced their first issues in 2010 and 2011 respectively) or those which were specific to a particular group or biome (such as Aquatic Invasions) were not included. We collected all articles relating to biological invasions that were published in the eight journals during 2009 and 2010 (the same period as the priorities were gathered) except letters to the editors, obituaries, book reviews and errata, which were not included in the assessment. Papers were classified using the main theme described in the title, or using the abstract when this was not clear. We attempted to classify all of the articles against the same thematic groups that had been identified from the priorities, but as many papers related to more than one priority area or covered different topics, the thematic groups were revised using an iterative process to better reflect the nature of the articles collected. Each paper was classified against only one main topic area (see online Appendix II: Journal article classifications for the data used in these analyses).

The priorities and research topics were compared using odds ratios (Bland and Altman 2000), and 95% confidence intervals, z statistics and p values were calculated. Odds ratios were calculated using the formula $OR = (a)/(449-a) / (b)/(789-b)$ whereby a= the number of times a priority is identified and b= the number of journal articles classified under that topic. An odds ratio of one suggests that the likelihood of the event occurring (in this case, of the topic being identified) is the same in both groups. Odds ratios were used here as the total number of priorities differed from the total number of articles retrieved.

Results

Stakeholder priorities

197 individuals responded to the different questionnaires (Table 1). Of these, 159 respondents provided a total of 449 individual priorities. Respondents represented a range of stakeholder groups; the main being researchers (40.5% of respondents providing priorities), practitioners (24.0%), and policy makers and advisors (20.3%). Respondents from other stakeholder groups such as volunteers or knowledge brokers accounted for 15.2% of respondents.

Nineteen broad priority categories or topics were identified (Figure 1). A quarter of all of the priorities identified by stakeholders (25.2%) related to the management of biological invasions. A further 16% related to information sharing, communication and collaboration, 9.1% related to education and awareness raising, 6.2% to economics, 5.1% to climate change and 4.9% each to impacts of invasive species and to synergies with climate change and other threat drivers.

When compared across stakeholder groups, the two most frequently identified priorities were the same for stakeholders working in management, policy and research; these were the management of invasive species, followed by information sharing, communication and collaboration (Figure 2). Despite being the most frequently identified, these topics represented varying proportions of the overall priorities within different stakeholder categories, representing 31.2% and 15.9% of manager priorities, 38.3% and 11.7% of policy stakeholder priorities and only 18.6% and 14.1% of researcher priorities respectively (Table 2). The order and relative proportions of subsequent priorities varied between stakeholder groups. Researchers identified priorities within each of the 19 topic areas, managers within 16, policy stakeholders within 15, whilst the ‘other stakeholders’ category only identified priorities within 13 of the topic areas. The ‘other stakeholders’ group most frequently identified information sharing, communication and collaboration as a key priority (27.5%), followed by education and awareness raising and the management of invasive species (17.4% each).

Table 1. The number and type of respondents each providing up to three priorities for invasive species science and management through questionnaires deployed at two events in 2009 and electronically in 2010.

Source	GB hard-copy questionnaires, 2009	Workshop at ECCB Conference, Prague, 2009	International electronic questionnaire, 2010	Total
Total no. respondents	41	18	138	197
No. providing priorities	37	17	104	158
No. working in research	9	14	41	64
No. working in practice	15	0	23	38
No. working in policy	11	3	18	32
No. of other stakeholders	2	0	22	24
Total priorities supplied:	98	48	303	449

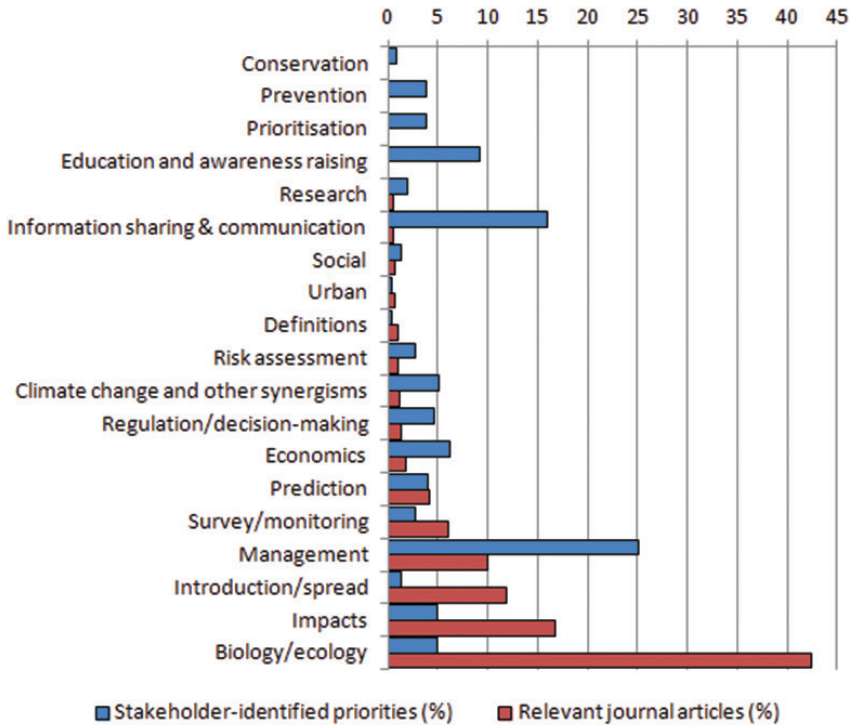


Figure 1. The relative proportions (%) of topics identified by stakeholders working with invasive species as priority areas for invasive species science and management compared to the topics of relevant journal articles published in eight journals over the same period (2009–2010).

Journal articles

789 articles of broad relevance to invasive species were identified from the eight journals during the two year period. Biological Invasions unsurprisingly published the highest number of articles deemed relevant to invasion biology as the only specialist journal included in the sample (545 articles). Diversity and Distributions published the second highest number (82), followed by Weed Research (75) and The Journal of Applied Ecology (48). Ecological Economics contained 12 articles, Journal of Environmental Management contained 11, Trends in Ecology and Evolution contained nine, and Conservation Evidence contained seven relevant articles. The majority of articles retrieved were original research articles.

Most journal articles related to the ecology or biology of invasive species (42.5%), the impacts of biological invasions (16.7%), or modes of introduction and spread (11.9%). The 79 management articles identified represented 10% of the sample. Approximately 6% of papers related to surveying or monitoring and 4.2% to prediction for invasive species. All other topics were the focus of less than 2% of articles in the sample.

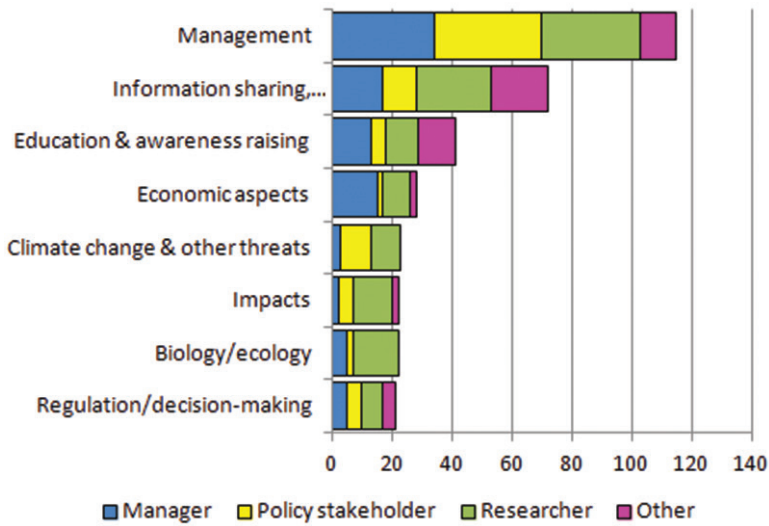


Figure 2. The eight main priority areas for invasive species science and management (each proposed twenty or more times) based on 344 of the 449 priorities identified by 158 stakeholders working with invasive species during 2009–2010 and depicted as absolute values broken down by stakeholder group. Detailed legend: Data plotted represents 94 of the total priorities provided by the 38 practitioners; 76 provided by the 32 policy stakeholders; 123 provided by the 64 researchers; and 51 provided by the 24 other stakeholders.

Comparison of topics

The greatest proportion of research papers related broadly to the biology and ecology of invasive species, whereas the greatest proportion of stakeholder priorities related to management (Figure 2). The odds ratio tests indicated that the proportion of topics identified as priorities by stakeholders were statistically different from the topics covered by journal articles for 14 out of 19 topics ($p \leq 0.05$; Table 3), indicating a mismatch. Education and awareness raising, prevention, prioritisation, information sharing and communication and conservation had the largest effect sizes, suggesting that they were under-represented in the literature when compared to the stakeholder priorities. Conservation, definitions, predictions, social issues and urban invasives were not significantly different with the 95% confidence interval crossing 1, suggesting that coverage of these topics by journals is roughly proportional to their identification as priorities; however, these topics represented only small values in both categories and so the odds ratios were likely to be closer to one.

Discussion

Our results showed an apparent mismatch between the topics relating to invasive species reported in journal articles and the priority areas for science and management

Table 2. Relative proportions (% to 1dp) of journal articles and stakeholder priorities (total and by individual stakeholder groups) addressing each of the 19 topics in invasive species science and management and collated during 2009–2010.

Topic	Relevant journal articles (%)	Total % of stakeholder-identified priorities	Manager %	Policy stakeholder %	Researcher %	Other %
Biology/ecology	42.5	4.9	4.6	2.1	8.5	0
Climate change and other synergisms	1.1	5.1	2.8	10.6	5.6	0
Conservation	0	0.9	0	0	2.3	0
Definitions	1.0	0.4	0	0	1.1	0
Economics	1.8	6.2	13.8	2.1	5.1	2.9
Education and awareness raising	0	9.1	11.9	5.3	6.2	17.4
Impacts	16.7	4.9	1.8	5.3	7.3	2.9
Information sharing, communication and collaboration	0.5	16.0	15.6	11.7	14.1	27.5
Introduction/spread	11.9	1.3	0	3.2	1.7	0
Management	10.0	25.2	31.2	38.3	18.6	17.4
Prediction	4.2	4.0	0.9	2.1	7.9	1.4
Prevention	0	3.8	1.8	3.2	2.8	10.1
Prioritisation	0	3.8	3.7	3.2	3.4	5.8
Regulation/decision-making	1.3	4.7	4.6	5.3	4.0	5.8
Research	0.5	2.0	1.8	1.1	2.8	1.4
Risk assessment	1.0	2.7	1.8	4.3	2.8	1.4
Social	0.6	1.3	0.9	2.1	1.1	1.4
Survey/monitoring	6.1	2.7	1.8	0	4.0	4.3
Urban	0.8	0.4	0.9	0	0.6	0

Table 3. Odds ratios with calculated 95% confidence intervals, z statistics and p values comparing the differences between the relative frequencies of stakeholder-identified priorities for invasive species science and management and the topics of journal articles published during the same period (2009–2010).

Topic	a) Number of times priority identified by stakeholders	b) Total number of other priorities	c) Number of relevant journal articles	d) Total number of other journal articles	Odds Ratio (a/b) / (c/d) to 2 dp	95% CI	z statistic	P
Climate change and other synergistic threats	23	426	9	780	4.68	2.15 to 10.20	3.88	P = 0.0001
Conservation	4	445	0	789	15.95	0.86 to 296.94	1.86	P = 0.0634
Definitions	2	447	8	781	0.44	0.09 to 2.06	1.05	P = 0.2961
Biology/ecology	22	427	335	454	0.07	0.05 to 0.11	11.56	P < 0.0001
Economics	28	421	14	775	3.68	1.92 to 7.07	3.92	P = 0.0001
Education and awareness raising	41	408	0	789	160.41	9.84 to 2614.46	3.57	P = 0.0004
Impacts	22	427	132	657	0.26	0.16 to 0.41	5.71	P < 0.0001
Information sharing, communication and collaboration	72	377	4	785	37.48	13.59 to 103.35	7.00	P < 0.0001
Introduction/spread	6	443	94	695	0.10	0.04 to 0.23	5.41	P < 0.0001
Management	113	336	79	710	3.02	2.20 to 4.14	6.87	P < 0.0001
Prediction	18	431	33	756	0.96	0.53 to 1.72	0.15	P = 0.8825
Prevention	17	432	0	789	63.89	3.83 to 1065.06	2.90	P = 0.0038
Prioritisation	17	432	0	789	63.89	3.83 to 1065.06	2.90	P = 0.0038
Regulation/decision-making	21	428	10	779	3.82	1.78 to 8.19	3.45	P = 0.0006
Research	9	440	4	785	4.01	1.23 to 13.11	2.30	P = 0.0214
Risk assessment	12	437	8	781	2.68	1.09 to 6.61	2.14	P = 0.0322
Social	6	443	5	784	2.12	0.64 to 7.00	1.24	P = 0.2158
Survey/monitoring	12	437	48	741	0.42	0.22 to 0.81	2.61	P = 0.0090
Urban	2	447	6	783	0.58	0.12 to 2.91	0.66	P = 0.5110

identified by stakeholders. This disparity, and in particular the lack of focus on management in the scientific literature, may be creating the perception that there is a gap between invasive species research and practice, supporting criticisms that research is not geared towards delivering practical solutions (e.g. Hulme 2006, Esler et al. 2010). However, there are several factors likely to influence this perceived mismatch.

Firstly, individual journal articles appeared to address multiple priority areas but were focused on specific species, sites or geographic regions, such as the introduction, spread and impacts of an individual species, whereas the priority areas identified by stakeholders in our sample were focused on defined topics such as 'management techniques' or 'surveying and monitoring'. This difference is likely to be due, at least in part, to the practicalities of undertaking field or laboratory research, necessitating greater focus and control. Baskerville (1997) identifies that scientists do not work or publish on the scale that ecological managers work in due to the focus on precision, and that the small temporal and spatial scales traditionally presented in the scientific literature have no context in management problems at the scales at which a manager must face. However, the increasing number of macro-ecological studies on invasive species may help to address this. Also, the stakeholders in our sample were not given a specific focus to consider when developing their priorities; the responses may have been more specific if we had asked for example about the priorities for their job, region or species of concern. Still, the information in journal articles may need to be unpacked and reassembled to better inform or address specific priorities.

Secondly, there is a clear justification for the focus on basic research on the ecology and population biology of invasive species. Fundamental research relating to both biology and management practices, as well as more advanced applied research such as modelling, are necessary to tackle the problems associated with invasive species and deliver practical solutions in the field (Simberloff et al. 2005; Van Acker 2009). Research into the population biology of invasive species is important in the management of established species and for policy formulation, but may be less useful when managing recently introduced species (Simberloff 2003). Despite the need for information on ecology and population biology to inform management, this is still lacking for many species (Simberloff 2003; McGeoch et al. 2010), with much research focused on a limited number of the most harmful species (Pyšek et al. 2008). Therefore a lack of information on the biology of a species is likely to constrain research into their management, but this may be naturally addressed as the field of invasion biology matures.

Thirdly, many journals focus on publishing articles that demonstrate novelty and broad interest, meaning that localised management studies may be seen as parochial and be rejected. Management actions are usually undertaken by non-research scientists and so the imperative to publish in academic journals is likely to be less, whilst negative results observed in the field may be difficult to get published but can have important implications for management (Sutherland et al. 2013). Other types of bias have been identified in ecological publishing that may affect the distribution of topics in the scientific literature, such as a bias towards the prevalent paradigms (Koricheva 2003). The inclusion of Conservation Evidence in this study aimed to capture articles relating

to invasive species management submitted by practitioner-led groups that may not be typically published in most scientific journals, although the number of papers relating to invasive species published during the study time period was low.

Finally, there may be a potential lag time between the identification of a priority and the reporting of research outcomes due to the time taken to mobilise funding and undertake the research. A comparison of stakeholder priorities collated several years prior to research outputs may provide a better reflection of the responsive nature of research.

Information for invasive species management

Biological invasions are by their nature multidisciplinary, and a wide range of subjects need to contribute to their successful management (Kühn et al. 2011, Williamson et al. 2011). For example, it may be important to understand human behaviours and motivations when trying to prevent releases of potentially invasive species by the public as part of a wider management programme. These forms of socially-derived data can be explicitly integrated into decision tools to guide invasive species management (Maguire 2004), although this approach has yet to be widely implemented. The need for research into biological invasions that crosses disciplinary boundaries has been identified elsewhere (e.g. Matzek et al. 2013). However, ecological journals cannot be expected to cover all of these topics as they have a clear purpose and audience, highlighting the crucial role for invasion-specific journals in helping to bring this information together. Whilst these topics may currently receive coverage in other discipline-specific journals that provide a more appropriate fit in terms of their scope, the journals may not be easily accessible to environmental workers with limited access to published research. There may also be lower awareness of papers published in other disciplines meaning that they are not identified or used by those that would benefit from the information they contain. Information sharing via colleague recommendation and sign-posting of relevant materials, as occurs through the IUCN SSC Invasive Species Specialist Group email list for example, may help to ensure that those working in the field become aware of other relevant materials (Bayliss et al. 2012). Information sharing was frequently identified as important by stakeholders, but this may have been influenced by the mechanisms used to capture to capture the data, as many of the responses came from an information sharing questionnaire, potentially introducing bias in the responses, and should be taken into account when interpreting the results. However, the information sharing category also included collaboration and cooperative working, which may also help to explain why it was so prominent as it may have included more than one priority from each individual.

Despite differences in the cultures and activities of different stakeholder groups, the two most frequently identified priority areas were the same for researchers, practitioners and policy stakeholders. This suggests that these areas, effective management and enhanced information sharing, communication and collaboration, require urgent attention. Although many of the stakeholder-identified priorities were addressed by research papers, important topics like education and awareness raising or prioritisa-

tion do not appear to be receiving sufficient coverage. Whilst these may not be the predominant tasks for scientists, an increasing focus on interdisciplinary projects like the Working for Water programme in South Africa may help to address the lack of coverage these topics currently receive. Although the largest proportion of respondents were researchers, their priorities still did not match the topics covered by journal articles, although they appear least different. However, they were the only stakeholders to identify as priorities some of the topics that were not significantly different from the topics of journal articles (i.e. conservation and definitions).

Although the management of invasive species was a key priority identified by stakeholders, we identified a lack of papers in the literature focused on the management of biological invasions, with most of those identified studying the impacts of invasive species control or eradication rather than its effectiveness. Other papers addressed management indirectly, for example by discussing the potential implications of a species' ecology on the effectiveness of management. The strong focus towards biology and ecology identified within the journal articles is likely to reflect the interests of the journals covered by this exercise; it is worth noting that the ecological journals contributed almost 87% of the total studies included in the analysis, and so despite efforts to include data from other journals to reduce the bias towards ecological studies, the greater volume of papers produced in these journals may also help to explain the focus towards ecological studies in the results. It may be interesting to repeat this exercise once more of the journals focused specifically on the field of biological invasions have had time to establish and mature.

Next steps

Kühn et al. (2011) identify seven broad areas in which our understanding relating to the management of invasive species could be improved. These include: social and political aspects of invasions; vector management for prevention; ballast water management; restoration; prevention tools e.g. risk analysis; legal measures; and rapid practical implementation of advances for prevention, eradication and control. We add that the sharing of experience of invasive species management, whether effective or otherwise, is important in providing an evidence base to evaluate and to inform practice. A greater focus on making management data available is required to provide the evidence needed to inform effective prevention and control. Any management data collected needs to be of a sufficient quality to allow robust analysis (Blossey 1999). In addition, we need to develop methods to analyse available management options. For example, an approach for analysing the costs and benefits of phytosanitary measures for use against quarantine pests (Kehlenbeck et al. 2012) could potentially be developed to analyse invasive species management actions more broadly.

Invasion biology as a discipline may need to find alternative mechanisms for collecting management information. There are many mechanisms available which can help, including publicly accessible newsletters and databases such as the Conservation

Evidence database and practitioner journal (www.conservationevidence.com), which includes case studies of invasive species management projects. Hoffman et al. (2011) used the Aliens newsletter of the IUCN Invasive Species Specialist Group to detail all of the unpublished ant eradication attempts they are aware of and include them in a synthesis of eradication attempts. However, scientists are also increasingly developing novel ways of disseminating their research findings to stakeholders. For example, attempts are being made to develop an early warning system for invasive species similar to those used in the European Union for animal health and food safety.

Of course, it is important to note that scientific information derived from research forms only one component of environmental management decisions. Previous research suggests that scientists are not keen to make decisive statements, preferring instead to articulate uncertainty and recommend other sources of information, whilst managers often have to make rapid decisions before all scientific information has been evaluated (Lach et al. 2003). Yet much of the information embedded in ecologically-focused research publications may be what is needed to inform policy and management but is incomprehensible in its current form, and may need collating and interpreting. The perceived gap between invasion biology research and practice may be best addressed through collaborative working and the translation of research findings into information accessible to end users. Scientists have a duty, particularly when their work is publically funded, to ensure that the scientific information they produce is not just published in journal articles but is explained to help resolve important policy questions (Lackey 2007). It may be that this is not a role for the scientists themselves, rather for specially trained knowledge facilitators (e.g. after Francis and Goodman 2010). Funders also have an important role in ensuring that applied research really is applied. Effective engagement is necessary to ensure that research is contextualised (Esler et al. 2010), whilst synthesis methods such as meta-analysis may help to address the difference in focal scales by combining data from multiple studies to inform decision making (Stewart 2010). Recent initiatives to collate primary research data to inform environmental management more broadly have advocated the use of synthesis methods such as systematic reviews or maps or synopses of conservation evidence (e.g. Pullin et al. 2009; Sutherland et al. 2013), and these may prove useful tools for invasive species management information.

Invasion biology, and ecology as a whole, may benefit from an independent organisation that draws scientific data together with other forms of relevant information to provide guidance on best practice, which could identify and steer funding towards the most pressing and topical questions. This would prove challenging as cohesion between stakeholders would be necessary, and this would depend on adopting a realistic and practical scale at which to operate. There is still a clear need for more basic research in invasion biology to provide the information necessary to elaborate more applied recommendations. Regardless of whether the priorities identified by stakeholders are addressed by research activities, there is a need to evaluate and share best practice. Traditional ecologically focused journals may not always provide the best forum for this, but as a community we need to ensure that information is being shared to enhance the integrated management of biological invasions.

Conclusions

Similarities in the priorities most frequently identified by different stakeholder groups suggest that there are broad topics that urgently need addressing, particularly in relation to the lack of research directly relevant to management or to sociological aspects of invasion biology such as education and awareness-raising. These may need to be addressed through research or through the evaluation and sharing of current experience to inform future practice. Whilst there are many topics still to be explored fully in invasion biology (e.g. Kühn et al. 2011, Williamson et al. 2011) that would benefit from new research, we consider that better use of existing information, much of which is not currently optimised to address pressing issues, would provide a sound basis for future research and management to build on.

As a community, we need to ensure that any research with practical applications to invasive species management addresses the needs of the stakeholders that ultimately stand to benefit from our science, either directly by undertaking targeted research with practical applications, or by ensuring that 'pure' biological and ecological research is translated or synthesised, either by researchers or by people trained for this purpose, so that the implications are better understood. By ensuring that the potential application of research is clearly expressed, and by finding ways to bridge the difference between research papers and stakeholder needs, efforts to control invasive species and the theoretical science of invasion biology will both be strengthened.

Acknowledgements

We particularly thank all of the individuals that provided us with their priorities for science and management. We also thank the British Ecological Society Invasive Species Group, the GB Non Native Species Secretariat, the Society for Conservation Biology European Section and the other individuals and organisations that kindly supported our workshops and questionnaires. We also thank Peter Mills and Jane Hill for feedback, and Ingolf Kühn and two anonymous referees whose comments helped greatly improve an earlier version of this manuscript.

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Appendix 1

Stakeholder priorities. (doi: 10.3897/neobiota.19.4897.app1) File format: Microsoft Comma Separated Value File (csv).

Explanation note: File containing stakeholder-identified priorities for invasive species science and management along with source from which they were obtained, stakeholder category (policy maker, practitioner, researcher or other) and the classification of the priority used for analysis.

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Citation: Bayliss HR, Stewart GB, Wilcox A, Randall NP (2013) A perceived gap between invasive species research and stakeholder priorities. *NeoBiota* 19: 67–82. doi: 10.3897/neobiota.19.4897 Stakeholder priorities. *NeoBiota* 19: 67–82. doi: 10.3897/neobiota.19.4897.app1

Appendix 2

Journal article classifications. (doi: 10.3897/neobiota.19.4897.app2) File format: Comma Separated Value File (csv).

Explanation note: File containing details of journal articles relevant to biological invasions included in the analysis, source journals and the main area of classification used for analysis.

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Citation: Bayliss HR, Stewart GB, Wilcox A, Randall NP (2013) A perceived gap between invasive species research and stakeholder priorities. *NeoBiota* 19: 67–82. doi: 10.3897/neobiota.19.4897 Journal article classifications. *NeoBiota* 19: 67–82. doi: 10.3897/neobiota.19.4897.app2

Communicating research with the public: evaluation of an invasive earthworm education program

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Academic editor: *Sven Bacher* | Received 11 February 2013 | Accepted 4 July 2013 | Published 11 October 2013

Citation: Cameron EK, Yuckin SJ, Bayne EM (2013) Communicating research with the public: evaluation of an invasive earthworm education program. *NeoBiota* 19: 83–97. doi: 10.3897/neobiota.19.4848

Abstract

Ecologists are increasingly encouraged by funding agencies and professional societies to communicate their research with the public. However, most receive relatively little training in how to do this effectively. Furthermore, evaluation of whether such an investment by ecologists actually achieves conservation objectives is rare. We created an education program, involving print, television, radio, and internet media, to increase awareness about earthworm invasions and to discourage anglers from dumping earthworm bait. Using pre- and post-surveys, we evaluated our program's success in reaching its target audience and in changing knowledge and behavior. Few participants (4.1%) recalled seeing the program material and knowledge of the fact that earthworms are non-native in Alberta remained low (15.8% before, 15.1% after). Further, after being told about the negative effects of earthworms in forests, 46.7% of the anglers surveyed stated they would not change their bait disposal behavior in the future, with many commenting that they did not believe earthworms could be harmful. These results highlight the importance of evaluating education programs, rather than assuming they are successful. Given many participants' doubts that earthworms have negative effects, both regulations and education may be needed to reduce earthworm introductions.

Keywords

angler, bait, behavior, evaluation, invasive species, non-native, public education

Introduction

Anthropogenic activities are causing unprecedented changes to the environment worldwide, leading to calls for ecologists to devote a greater amount of their time to communicating research findings with the public and policy makers (Lubchenco 1998, Holdren 2008). Many ecologists now consider such communication to be an important part of their careers (Pace et al. 2010). Public communication, and even advocacy, is viewed by some as a social responsibility of scientists (Lovejoy 1989, Noss 2007, Nelson and Vucetich 2009). This sense of duty may be a particularly important source of motivation for senior researchers, while junior scientists appear to be driven by enjoyment and personal satisfaction as well (Martin-Sempere et al. 2008). Unfortunately, regardless of their motivation for participation in public outreach, many scientists' academic training provides them with little opportunity to learn effective methods for communicating with the public. Further, scientists often do not view public communication as a core aspect of their work and may believe it has neutral or negative impacts on promotion because of the time it takes away from research (Gascoigne and Metcalfe 1997, Martin-Sempere et al. 2008).

Professional organizations such as the American Association for the Advancement of Science and the International Council for Science include enhancing communication among scientists and the public and improving science education as key goals for their organizations (AAAS 2012, ICSU 2012). Funding agencies including the National Science Foundation in the United States, and the National Science and Engineering Research Council in Canada have also attempted to promote public communication activities by including criteria relating to outreach efforts in their grant evaluation processes (Holbrook 2005). These types of initiatives should encourage public communication and education to be viewed as an integral component of scientific research. However, the high rates of failure (40–50%) estimated for public education campaigns (Ostergaard 2002) suggest that evaluation of the success of public communication efforts should be a critical part of outreach activities carried out by ecologists. Researchers' assumptions about a program's success are not sufficient.

Ideally, evaluation should include both formative evaluation, which is used to improve the program while it is being designed or run, and summative evaluation, which is used to determine if the program has achieved its objectives once it is complete (Carleton-Hug and Hug 2010). Yet, assessment of the success of public outreach efforts, whether conducted by academic scientists or others, is often overlooked. In a review of articles published from 1993 to 2008, only 20 evaluations of environmental education programs were found in the three leading environmental education journals (Carleton-Hug and Hug 2010). Lack of funding or time, inexperience with survey design, and fear of negative consequences if the program was unsuccessful are key reasons evaluations are not included in programs (Bitgood 1996, Carleton-Hug and Hug 2010).

Invasive species are often the focus of public education programs (e.g., Alexander and Lee 2010, Hickey 2010, Jordan et al. 2011, Sya et al. 2009) because humans can act as key vectors for invasive spread. Our research on earthworm spread in Alberta,

Canada (Cameron et al. 2008, Cameron et al. 2007) led us to work with various agencies to develop a public education program about the effects of earthworms in Alberta. Such non-charismatic species may pose particular challenges for education and management, as their invasions may receive little public attention or initially proceed undetected. In part due to the limited availability of information on earthworm invasions, “vegetation change facilitated by earthworms in North American forests” was recently identified by leading scientists as one of the top 15 global conservation issues (Sutherland et al. 2011). In northern forests, earthworms can mix organic and mineral soil horizons (Alban and Berry 1994), increase leaching of nitrate (Costello and Lamberti 2008), and alter below- and above-ground plant and animal communities (Gundale 2002, Hale et al. 2006, Eisenhauer et al. 2007).

European earthworms were accidentally introduced into North America in dry ship ballast, on plants, and in soils brought from Europe, and intentionally in agricultural areas to improve soil conditions (Gates 1970, 1982, Hendrix and Bohlen 2002). Invasions of earthworms are now occurring in remote areas across North America (Gates 1970, Gundale et al. 2005, Cameron et al. 2007), including northern forests that were previously glaciated and have no native earthworms (Gates 1970, 1982, Hendrix and Bohlen 2002). Earthworm invasion into these forests is facilitated by human activities, with dispersal of smaller earthworm species occurring along roadways in transported soil or in vehicle tire treads (Dymond et al. 1997, Cameron et al. 2007). Larger earthworm species are mainly invading near lakes, where anglers release them after using them as bait (Cameron et al. 2007, Keller et al. 2007, Kilian et al. 2012). Management of invasive earthworms in northern forests has focused on trying to prevent their introduction since there is no known method of control once populations are established (Callaham et al. 2006).

Education programs about the ecological effects of earthworms have emphasized the need to stop people from discarding live earthworm bait in areas where earthworms may establish new populations, although most efforts are on a small scale (Keller et al. 2007). The largest program in the United States, the Great Lakes Worm Watch (developed by the Natural Resources Research Institute), has done this, in part, through the distribution of more than 1500 posters and fact sheets to bait shops, nature centers, and park visitor centers (Callaham et al. 2006). Callaham et al. (2006) describe public response to this educational campaign as favorable and state that the message to stop dumping bait has been well received. However, there does not appear to have been any formal published evaluation of the success of this program, or other earthworm-related programs, at informing the general public and altering behavior. If scientists are to effectively raise public awareness, more critical and objective evaluations of the success of public education projects are required.

We developed an education program and examined its effectiveness by testing whether changes occurred in knowledge or behavior after program implementation. Using pre- and post- surveys, we evaluated whether anglers had encountered program materials, their level of knowledge about earthworm invasions, and their attitudes towards changing their method of disposing of earthworm bait. An increase in awareness

of the fact that earthworms are not historically found in Alberta and a decrease in the number of anglers discarding bait on land or in water would suggest that our program was effective.

Materials and methods

Education program

Our education program, the “Alberta Worm Invasion Project”, was designed to increase public awareness of earthworm invasions in forests. We began developing this program in 2009, after conducting several years of research which indicated humans are a key vector involved in the spread of earthworms in Alberta. The key messages of the program were that earthworms are invasive and can harm plants and animals in forests, and the public can help prevent earthworm introduction by not dumping bait. The program’s overall goals were thus to increase knowledge about earthworm invasions but also to change the behavior of anglers who release live earthworm bait into forests and lakes and convince them instead to save their bait or dispose of it in the garbage. It included five types of media: posters placed in bait shops, a website, two radio interviews, two televised informational clips, and two magazine articles (Table 1).

Posters were sent to 250 bait stores throughout northern Alberta, Edmonton, and Calgary by a bait distributor in 2010 (Figure 1). The main species distributed commercially in Alberta is *Lumbricus terrestris*, which is native to Europe but invasive in North America (Gates 1972). Bait stores included large specialized shops as well as gas stations that sell bait and approximately 100 of the stores accepted the posters and displayed them. The posters included a link to our website (<http://worms.biology.ualberta.ca>), which was also developed in 2010. This site can also be found if the phrase “worm invasion” or “Alberta worms” is searched in Google. On the website, five pages provide detailed information on earthworm spread, impacts on forests, ecological groups, how to avoid introduction, and an overview of our current research.

Let’s Go Outdoors Radio aired two interviews about the spread of earthworms and also created two television clips in partnership with the Alberta Conservation Association (ACA). In the spring of 2010 and 2011, the radio interviews ran during a weekend

Table 1. Educational program media and estimated audience sizes. Percentages in brackets are the audience sizes expressed as a proportion of the total population of Alberta.

Media	Audience
Conservation magazine article	40,000 (3.8%)
New Trail magazine article	140,000 (1.1%)
Posters	~100 stores, number of people unknown
Radio interviews	100,000 in each of 2010 and 2011 (2.7%)
TV clips	600,000 in each of 2010 and 2011 (16%)
Website	Not available

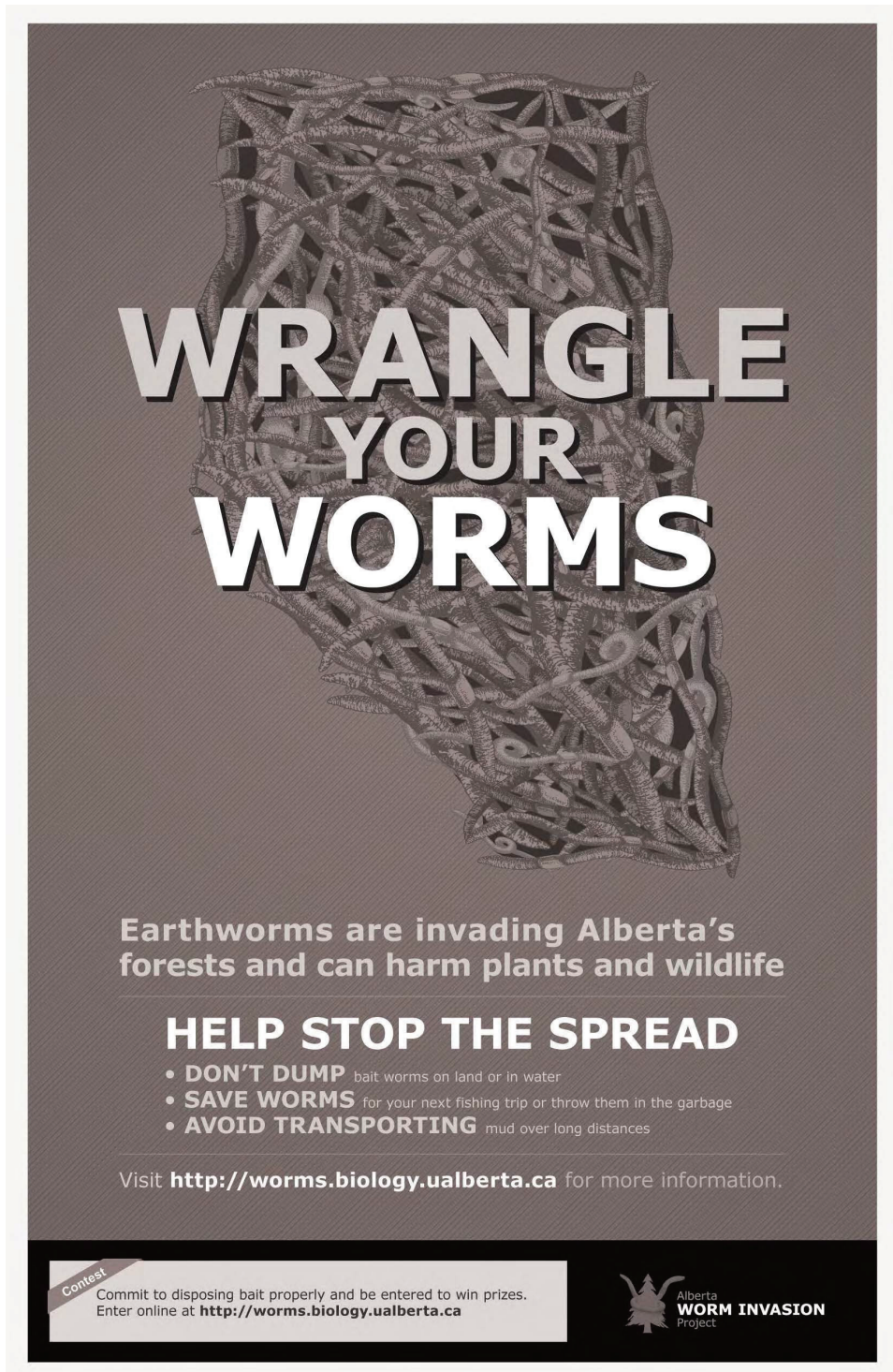


Figure 1. Poster distributed to bait stores as part of our earthworm education program.

show in 16 communities across Alberta with an expected audience of 100,000 in each case. One television clip was played in 2010 and the other in 2011. Each was played 48 times over the course of two weeks in early summer, and was estimated to have an audience of 600,000 viewers. A magazine article was published in the ACA's *Conservation Magazine* (Cameron 2010) and in the University of Alberta's alumni magazine *New Trail* (Habib 2011). These magazines have readerships of approximately 40,000 and 140,000, respectively. *Conservation Magazine* is targeted to anglers and hunters, while *New Trail* reaches a larger number of Albertans but is not targeted to anglers. All interviews, television clips, and articles included the information that earthworms are not native to Alberta's boreal forest and can be spread by anglers dumping bait, as well as some discussion of the effects of earthworms in forests.

Program evaluation

In-person oral pre- and post-interviews were used to evaluate our invasive earthworm education program. All individuals surveyed were a minimum of eighteen years old. The pre-surveys were carried out in the summer of 2009, with the ACA conducting 213 surveys and Alberta Sustainable Resource Development (ASRD) conducting 2018 surveys as part of their larger creel surveys of anglers. The post-surveys took place during the summers of 2011 and 2012. The ACA conducted 15 surveys and ASRD conducted 150 surveys in 2011, while we carried out a further 245 surveys in 2012. All surveys were performed at lakes across Alberta where participants were engaged in fishing activities. ACA conducted surveys in northern Alberta and ASRD conducted surveys in south-central Alberta, while our surveys in 2012 were carried out at previously sampled lakes across Alberta (Figure 2). In addition to these before-after surveys, we carried out a further 346 surveys in March 2011 at the Edmonton Boat and Sportsmen Show to obtain additional information on program effectiveness. Because the anglers at the show may have represented a different population of anglers and these surveys were conducted prior to the airing of the second television and radio clips, this data was not compared directly to the 2009 surveys but instead was examined qualitatively.

The 2009 pre-survey contained three questions (Table 2), which addressed participants' use of earthworm bait, how they dispose of bait, and their awareness that earthworms are not native to Alberta's forests. The surveys in 2011/12 included the same three questions and one to three additional questions (Table 2). The additional questions examined whether participants had seen material from our education program and whether they had changed their bait disposal behavior or would be willing to change their behavior in the future. If the participant was not using earthworm bait, only the questions examining if they knew earthworms were historically found in Alberta and if they had seen any information from our program were asked (questions 1 and 4 in Table 2). If they did use earthworm bait and had seen the information, they were also asked if the information caused them to change their bait use (question 6 in Table 2). If they had not seen the information but used earthworm bait, they were asked if they would change

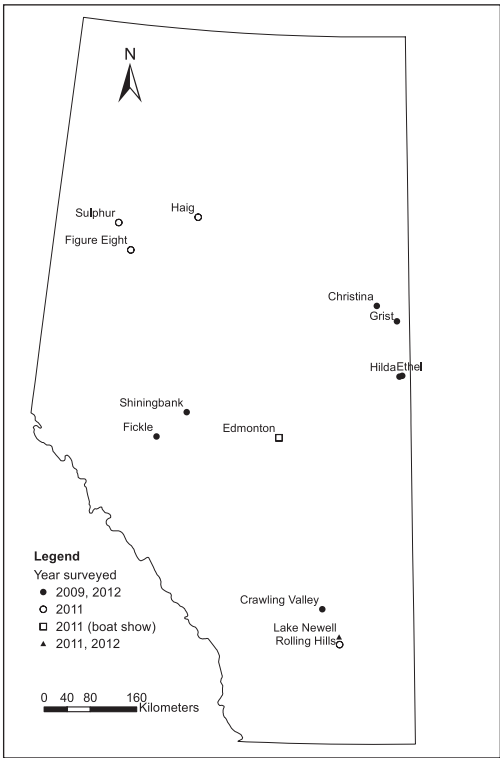


Figure 2. Locations of surveys across Alberta, with ● representing lakes surveyed in 2009 and 2012, ○ = lakes surveyed in 2011, □ = Edmonton Boat and Sportsman Show in 2011, and ▲ = lakes surveyed in 2011 and 2012.

Table 2. Angler survey questions. Questions 1 to 3 were asked in 2009 and 2011/12, while questions 4 to 7 were only asked in 2011/12.

Question	Closed answer options
1. If you use earthworm bait, where do you get it from? (if a), go to 3)	a) did not use b) caught near fishing location c) brought from home
2. At the end of the day, what do you do with your leftover earthworm bait?	a) release in lake, b) release on land, c) dispose of in trash, d) save for next trip
3. Do you think earthworms were historically found in this region?	a) yes, b) no, c) do not know
4. In the past year, have you seen any information about earthworm invasions in Alberta? (if b), go to 7)	a) yes, b) no
5. Where was this information from? If other, provide source.	a) posters, b) TV commercial, c) website, d) article, e) other
6. If you have heard about earthworm invasions, has it changed your use of bait?	a) yes, b) no
7. Knowing that earthworms are invasive to boreal forests in Alberta and can negatively affect plants and animals, will you change your use of earthworm bait?	a) yes, b) no

their bait use (question 7 in Table 1). In the 2012 surveys and the Edmonton Boat and Sportsmen Show surveys, additional time was available as our questions were not part of a lengthy creel survey. Therefore, we asked anglers if they would change their bait use if they ever use bait, rather than asking question 7 only to people using bait that day.

Data analysis

Chi-squared tests were used to examine whether bait use, bait disposal, and angler knowledge changed after implementation of the program. In all analyses, the independent variable was whether the survey was conducted before or after the program (i.e., 2009 vs. 2011/12). We tested whether use of earthworm bait (the proportion of anglers who used earthworm bait) changed after the program was implemented. We also examined whether the location where bait was acquired (dependent variable = proportion bringing bait from home versus digging it up at the lake) and bait disposal (dependent variable = proportion who saved bait/discarded it in the trash versus discarded it on land/water) changed after the program. Changes in awareness of earthworm invasions after the program was implemented were also examined. In this analysis, the dependent variable was the proportion of anglers who knew earthworms were not native to Alberta. Analyses were performed in Stata version 11 and were considered statistically significant at $P < 0.05$.

Results

Before-after surveys

A similar proportion of anglers reported using earthworm bait on the day they were surveyed in 2009 (9.2% SE 0.61) and 2011/12 (7.6% SE 1.3) according to a chi-squared test ($\chi^2 = 1.13$, $P = 0.29$). In both years, most people brought their bait from home rather than digging it up at their fishing location. However, the proportion of people bringing bait from home was significantly higher in 2009 at 99% (SE 0.69) than in 2011/12 at 83.9% (SE 6.7) ($\chi^2 = 21.48$, $P < 0.001$).

We used a chi-squared test to compare methods of disposing of earthworms that could contribute to their spread (releasing them in the lake or on land) versus disposal of earthworms that could prevent anglers from contributing to their spread (disposing of them in the trash or saving them for the next trip). There was a significant difference between 2009 and 2011/12 ($\chi^2 = 6.21$, $P = 0.013$), with 39.2% (SE 3.4) of participants in 2009 and 62.5% (SE 8.7) in 2011/12 disposing of earthworm bait in lakes or on land (Figure 3).

Before implementation of our education program, 15.8% (SE 0.77) of anglers were aware that earthworms were not historically found in Alberta, compared to 15.1% (SE 1.8) of anglers in 2011/12. This difference was not significant ($\chi^2 = 0.13$, $P = 0.72$).

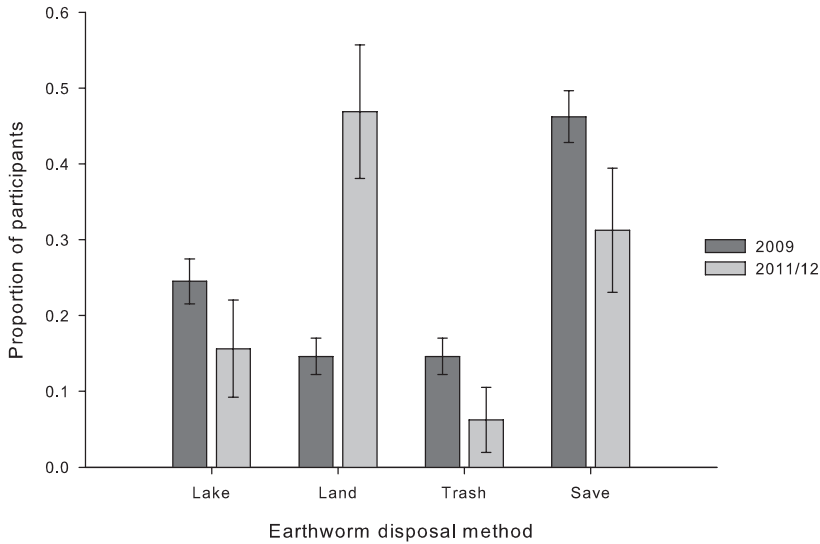


Figure 3. Proportion of participants (\pm SE) using different earthworm bait disposal methods (release in lake, release on land, put in trash, save for next trip) in 2009 ($n=212$) and 2011/12 ($n=32$).

Qualitative results (boat show and 2011/12 lake surveys)

When the 2011/12 survey respondents (including both those at the boat show and lakes; $n = 756$) were asked if they had seen any information about earthworm invasions in Alberta during the past year, only 31 people, or 4.1%, indicated that they had. Most of these participants saw an article on earthworm invasions (35.5%) or a TV clip (22.6%). The remainder received information from the website (16.1%), a poster (12.9%), another person (6.5%), the radio (3.2% - 1 person), or could not remember where they saw the information (1 person).

Only eight of the participants who had seen information from our program were users of earthworm bait. Of these eight, only three indicated they had changed their behavior by reducing their use of earthworm bait or putting leftover bait in the garbage. A total of 276 participants used earthworm bait at least occasionally and had not seen the education program material. When they were asked if they would change their use of earthworms as bait knowing that they are invasive and can negatively affect plants and animals, 46.7% responded that they would not.

Discussion

Program's level of success

Despite using a variety of forms of media, including print, television, radio, and internet, our program appeared to reach only a small number of anglers and to have a

limited effect on knowledge and behavior. Only 4.1% of the surveyed anglers reported seeing information from our program. This low proportion suggests that our program might have benefitted from a more detailed preliminary examination of whether the media employed were likely to successfully reach our target audience. No increase in awareness of earthworm invasions was observed, with 15.8% and 15.1% of respondents before and after our program, respectively, stating correctly that earthworms were not historically found in Alberta. This level of awareness is similar to that encountered in surveys in New York, where 17% of people were aware that earthworms were exotic invasive species (Seidl and Klepeis 2011). Overall, our results emphasize the importance of conducting evaluations to assess the effectiveness of public awareness programs, rather than assuming programs have been successful based on the amount of program literature distributed or the expected audience size for various media.

It has been suggested scientists should devote one tenth of their professional time to outreach efforts and other activities intended to increase the societal benefits of science and technology research (Holdren 2008). We estimate that our program took approximately 140 hours for one of us to initiate over the course of three years, which is considerably less than this suggested amount but still represents a substantial time commitment. Given the limited change in awareness, it could be questioned whether these types of activities are a worthwhile investment for ecologists. However, it is likely that the amount of time and money (~\$5000 which was spent mainly on website design, poster design, and poster printing, as we were not charged for television and radio clips) required to set up this program was less than would have been required by a conservation or government group. Although we lacked experience with public education programs, we were already familiar with research on earthworm invasions and had resources (e.g., pictures used on the website and magazine articles) available from previous work. Rather than viewing the program's limited success as an indication that academic scientists should not attempt this type of work, it could instead be seen as providing evidence of the importance of evaluating programs while carrying them out. Conservation education programs which include some type of formative evaluation are more likely to be successful (Jacobson and McDuff 1997; Norris and Jacobson 1998).

Effectiveness of media types

Some types of media were more effective than others at reaching our target audience. The articles (35.5%) and television clips (22.6%) were the forms of information most often encountered. Use of mass media such as television and radio has been identified as a factor contributing to the success of conservation programs (Jacobson and McDuff 1997). Fewer participants reported seeing our poster or website and only one mentioned the radio interview. Previous research on bait use recommended that bait stores would be an ideal location for placing signs or other sources of information about earthworm invasions (Keller et al. 2007). Our survey at the Edmonton Boat and Sportsmen Show also confirmed that many anglers do purchase their bait and thus

targeting bait stores with program materials does seem to be a reasonable approach. However, signs had similarly limited effects on behavior in a study on bear-proof garbage containers (Baruch-Mordo et al. 2011). Although we consulted with other biologists and environmental program coordinators when designing our program, a more interdisciplinary approach involving greater initial consultation with other disciplines (e.g., psychology, education, business) might have improved our program's success but would also have required a substantially greater investment of our time.

Several alternative methods of delivering program material, which we have been developing through consultation with researchers in sociology and education, may be more effective than the strategies used in our program thus far. First, warning labels could be placed on bait container lids to reach anglers in bait stores. Because anglers should see these labels whenever they use their bait, stickers are less likely to be overlooked than posters on bait fridges. We conducted a small pilot study to test this idea, in which we placed stickers on 5000 containers in 2012. Because we wanted to understand whether bait disposal behavior changed when stickers were present, we enlisted the assistance of store cashiers to collect contact information of bait purchasers. We then surveyed these anglers two weeks after their purchase and 75% stated they saved their bait or put it in the trash, compared to 61% and 37% in our before-after surveys. Unfortunately, the response rate was extremely low with only 12 people responding to the survey, although a greater number left their email addresses. Nonetheless, these results suggest this strategy deserves further examination. Secondly, involvement in citizen science programs can lead to increased knowledge among the public (Jordan et al. 2011). Thus, we have begun collaborating with researchers in the Faculty of Education at the University of Alberta to develop a smartphone application that allows students and the public to participate in data collection on earthworm distributions in Alberta. Thirdly, two participants in our surveys indicated they heard about our program from other anglers, rather than by directly encountering program material. Research on behavioral choices indicates that knowing others are behaving in a particular way can strongly encourage people to conform to the same behavior, regardless of their own level of environmental awareness (Clayton and Myers 2009; Michel-Guillou and Moser 2006). Consequently, encouraging anglers who are active in anglers' associations or work in fishing stores to pass information on to other anglers might lead to greater changes in behavior.

Behavioral changes

There was no decrease in bait abandonment, with the proportion of anglers disposing of bait on land or water increasing from 39% in 2009 to 63% in 2011/12. This was driven largely by an increase in bait disposal on land, but it is not clear why such an increase would have occurred. Only three out of the eight people who used earthworm bait and also saw our program material stated that they had changed their approach to disposing of bait as a result. Furthermore, many people (46.7%) who did not see our

material indicated they would not change their earthworm bait use/disposal after being told that earthworms were not native to the area and could harm plants and animals. Although we did not ask participants for comments on why they would or would not change their bait disposal, 30% of the people at the Edmonton Boat and Sportsmen Show who stated that they would not change commented that they did not believe earthworms could be a problem. Similar to this, almost 85% of residents surveyed in a study in New York moderately or strongly agreed that earthworms have a positive impact on plants (Seidl and Klepeis 2011). Many adults have likely heard or been taught about the benefits of earthworms for soil in gardens and agricultural systems. The reputation of earthworms as beneficial therefore appears to present an additional challenge for management efforts. On the other hand, even when people are aware that species are invasive, they may be unwilling to change their behavior. In a recent survey on attitudes towards invasive species, almost 30% of individuals were willing to introduce non-native species to an area if they would personally benefit (Garcia-Llorente et al. 2008).

Conclusions

The limited impact of our invasive earthworm education program highlights the importance of evaluating conservation programs. Formative evaluation is particularly essential as it allows for the improvement of programs while they are being carried out. A more interdisciplinary approach to program design may also lead to greater program success. Our survey confirmed that anglers are a source of earthworm introduction in Alberta, and therefore efforts to target anglers are needed if a reduction in spread of earthworms is desired. Increased access to artificial lures or proper disposal methods, such as labeled trash cans at boat launches, could make it easier for anglers to behave responsibly (Seidl and Klepeis 2011). However, evaluations of other education campaigns have concluded that regulations or regulations combined with education are more effective than education alone at bringing about behavioral changes (Baruch-Mordo et al. 2011). The non-charismatic nature of earthworms and the disbelief many respondents expressed upon being told earthworms could be harmful in forests suggest that regulations restricting bait dumping or bait sales, as have been implemented in other jurisdictions (Callaham et al. 2006, Kilian et al. 2012), are likely needed for a significant reduction to occur in earthworm introductions.

Acknowledgments

We particularly thank the Alberta Conservation Association (ACA), L. Winkel and D. Park of Alberta Sustainable Resource Development, J. Junas-Grant, A. Komaratat, R. Kong, J. Kummer, S. Pearce Meijerink and K. Wyering for their data collection efforts. Thanks to B. Cameron, K. Cameron, M. Short, Alberta Ecotrust, and the

ACA for assistance with program development. The manuscript benefited from comments by I. Barrio, T. Bao, G. Bueno, and S. Nyanumba. This research was funded by the ACA, Alberta Ecotrust, a National Sciences and Engineering Research Council (NSERC) Vanier Canada Graduate Scholarship to E.K.C., and a NSERC operating grant to E.M.B. This research was conducted under permits # Pro00032415 and # Pro00012802 from the Research Ethics Board at the University of Alberta.

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