

Comparing functional similarity between a native and an alien slug in temperate rain forests of British Columbia

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Academic editor: W. Nentwig | Received 19 July 2014 | Accepted 28 November 2014 | Published 14 April 2015

Citation: Rodriguez-Cabal MA, Gibbons TC, PM, Barrios-Garcia MN, Crutsinger GM (2015) Comparing functional similarity between a native and an alien slug in temperate rain forests of British Columbia. NeoBiota 25: 1–14. doi: 10.3897/neobiota.25.8316

Abstract

The impacts of invasive alien species are greatest when they become dominant members of a community, introduce novel traits, and displace native species. Invasions by alien mollusks represent a novel context by which to compare trait differences between generalist native and introduced herbivores in terrestrial ecosystems. Here, we determined the abundance, habitat, feeding preferences, as well as the metabolic rate of the native Pacific banana slug (*Ariolimax columbianus*) and the alien black slug (*Arion rufus*) in the coastal forests of British Columbia, Canada. Through a series of observational and experimental studies, we found that alien slugs are more abundant, differ in their habitat preferences, and consumed more fungi (mushrooms) than native banana slugs. Conversely, in an enclosures experiment we found that herbivory damage by native slugs was higher compared to enclosures with alien only and control enclosures. Finally, metabolic rates were similar for both slug species. These results suggest that alien black slugs possess a suite of traits that make them functionally different from native banana slugs.

Keywords

European black slug, Gastropoda, generalist herbivores, invasive species, Pacific banana slug, metabolic rate

Introduction

The rampant loss of biodiversity and the spread of alien invasive species are pervasive components of global change (Vitousek et al. 1997, Chapin et al. 2000, Simberloff et al. 2012), and a major priority in ecology is to understand the effects of biological invasions on native communities and ecosystems (Wardle et al. 2011, Simberloff et al. 2012). In general, the impacts of invasive species on native species are greatest when they introduce novel traits and become dominant members of a community. For example, alien herbivores can modify plant communities by directly affecting plant survival, growth and fitness through browsing, grazing and trampling (Crawley 1986, Barrios-Garcia et al. 2012) or through indirect effects by altering nutrient cycles, primary productivity, disturbance regimens, and disrupting mutualisms (Williamson 1996, Mack and D'Antonio 1998, Rodriguez-Cabal et al. 2013). Yet, it remains unclear whether alien herbivores are introducing novel traits and are, therefore, functionally different than native herbivores or whether alien herbivores are performing similarly to native herbivores, but simply have higher abundances. Moreover, while extensive research has focused on the impacts of vertebrate herbivores on ecosystems (Wardle et al. 2001, Husheer et al. 2003, Côté et al. 2004), much less is known about the mechanisms that make invertebrate herbivores successful invaders.

Invasion by alien mollusks, and slugs in particular, represents a novel context in which to compare native and introduced herbivores. Alien slugs have been identified as a global pest in a wide range of managed ecosystems, from large agricultural fields to urban backyard gardens (South 1992, Joe and Daehler 2008). In addition, slugs can play important roles in natural ecosystems and crops through their effects on seedling survival, plant fitness, vegetation biomass (Hulme 1996, Hanley 1998, Joe and Daehler 2008, Strauss et al. 2009), the facilitation of alien plant species (Sessions and Kelly 2002), the acceleration of litter decomposition (Jennings and Barkham 1979), and altering litter invertebrate populations (Ferguson 2004). Despite the potential impacts of slug invasion, little is known about whether slugs are introducing novel traits compared to native slug communities. There is limited information available for pre-invasion communities and, to our knowledge, there have been no detailed comparisons of the characteristics of native and alien slugs or their impacts on native plant communities (but see Ryser et al. 2011, Knop and Reusser 2012, Blattmann et al. 2013). Such functional comparisons are increasingly important given that native terrestrial mollusks have the highest number of documented extinctions of any major taxonomic group, most of which can be directly linked to introduced mollusk species (Lydeard et al. 2004, Nash 2004).

The Pacific banana slug (*Ariolimax columbianus*, Gould in A. Binney, 1851) is a charismatic species that is native to the Pacific Northwest Region of North America, with a distribution that extends from the central coast of California through Alaska (Harper 1988). The Pacific banana slug is the second largest slug in the world (*ca* 260 mm long), lives for several years, and is the most common native slug species in coastal British Columbia (BC) forests (Gordon 1994, Forsyth 2004). Little is known about

the specific diet of banana slugs or most native slugs in BC forests (Forsyth 2004), but they are presumed to be generalist herbivores (Cates and Orians 1975) that feed upon a variety of plant species, berries (Richter 1976, Gervais et al. 1998), organic detritus and fungi (Forsyth 2004). On the other hand, *Arion rufus* L. (1758, hereafter referred to as 'black slugs') is native to western and central Europe (Forsyth 2004). Black slugs have been observed in BC since 1941 and have become the most common slug found in the southern portion of the province (Forsyth, 2004). Black slugs tend to be smaller than banana slugs, growing up to ~180 mm long, but appear to be similar in their generalist diet, feeding upon animal feces, carrion, fungi, lichens and plants (Forsyth, 2004). Unlike the banana slugs, black slugs live for only a single year (Hamilton and Wellington 1981). These alien slugs are also known to be aggressive towards other slugs (Forsyth 2004), though little work has been done on the impacts of the invasive slugs on BC flora or fauna, and whether they have negative effects on the abundance of Pacific banana slugs is unknown.

Despite the large size and high visibility of both banana and black slugs, little is known about the ecological importance of either species for forest ecosystems. Moreover, the ecological impacts of European black slug invasion remain unknown for coastal rain forests, despite being highly abundant in the understory (Rodriguez-Cabal pers. obs.). Here, we use observational and experimental studies to compare native Pacific banana slugs and the alien black slugs in the coastal forest of BC. Specifically, we ask whether there are differences between the banana slug and the alien black slug in (1) abundance and (2) feeding preferences. Next, we compare the metabolic rate as a key trait that might vary between native and alien slugs for two main reasons. First, we suspected that there could be potential differences in thermal tolerance between the two species as a result of differences in their coloration (yellow versus black), which, in turn, could allow for differences in foraging time. Second, we wanted to determine whether slugs have different metabolic rates that could explain any possible differences in food consumption rates between the two species. These comparisons allow us to begin to understand the general ecological role of slugs in coastal forest ecosystems and determine whether alien slugs are playing functionally similar roles as native slugs, thereby providing general insight into the effects of slug invasions.

Methods

Study site

We conducted this work between June and August of 2012 at the Malcolm Knapp Research Forest (MKRF), which is a 5,157 ha research forest in Maple Ridge, British Columbia, Canada (49°16'N, 122°34'W). The most common overstory trees in this forest are evergreen red cedar (*Thuja plicata*), Douglas fir (*Pseudotsuga menziesii*), and the western hemlock (*Tsuga heterophylla*). The understory is dominated by Pacific blackberry (*Rubus ursinus*), salmonberry (*Rubus spectabilis*), red Huckleberry (*Vaccini-*

um parvifolium), salal (*Gaultheria shallon*), Pacific oak fern (*Gymnocarpium dryopteris*), common sword fern (*Polystichum munitum*), as well as a diverse assemblage of lichens and bryophytes. Forest harvesting has occurred in MKRF since 1949, which has led to a range of age classes from clear cuts, second and third growth, some small patches of > 400 year old growth forest, and different combinations of overstory species.

Slug abundance

We compared banana and black slug abundances using pitfall traps at 15 sites throughout MKRF in four different types of dominant forest overstory: cedar ($n = 4$ sites), Douglas fir ($n = 4$ sites), hemlock ($n = 4$ sites) and clear cuts between 2-5 years old ($n = 3$ sites). Forest sites were selected to encompass a range of forest ages at the study site (from 60 to 200 years old) and were at least 1 ha in size. Within each site, we placed a 3×3 grid of pitfall traps spaced 5 m apart (total area of 100 m²). At each point of the grid, we placed a single pitfall trap made of SOLO® Red Cups (473 mL, 9.86 cm in diameter) containing a sponge soaked with beer (typically a light Canadian-brand lager) as beer is attractive for snails and slugs (Edwards 1991). Pitfall traps were buried flush with the ground. We covered each trap with elevated wooden cover boards (25 x 25 cm). Because beer traps may attract one species more than the other, beer traps may yield biased estimates of natural slug abundance. We therefore used wooden cover boards to provide a combined estimate of slug abundance. Because the wooden boards were covering the beer traps we counted the total number of slugs using the combined methods. The use of both methods, beer traps and wooden boards, might reduce the bias of any one sampling method. After 24-hours, we counted and identified all of the slugs in the beer traps and under the cover boards. We repeated the sampling once per month ($n = 3$) during the summer (June to August) on days with similar weather conditions. Captured live slugs were brought back to the lab for use in cafeteria experiments (see below).

We used Generalized Linear Models (GLM) using a Poisson distribution with a log link function to compare slug abundances by habitat type (cedar, Douglas fir, hemlock and clear cuts) and slug type (native vs. alien). We calculated slug abundance as cumulative number of slugs (summing the total number of slugs captured at each site during the summer) in each environment. Our objective of slug sampling was to obtain relative estimates of their abundance over space and time, so we considered the combined use of beer traps and wooden boards sampling method as adequate for this purpose. As we removed the captured slugs in previous censuses, we were not re-sampling the same individuals.

Cafeteria experiments

A total of 189 slugs (90 native and 99 alien) were collected from MKRF to assess their feeding rates for fruits of common native understory plant species and fungal fruit-

ing bodies (i.e. mushrooms). Individual slugs were kept in separate plastic containers (33.8 cm × 21.6 cm × 11.9 cm, Rubbermaid, Inc., Huntersville, NC, USA) on a standard diet of lettuce (*Lactuca sativa*) for 72 hours prior to feeding trials. A paper towel dampened with water was placed at the bottom of the container to prevent desiccation. We then collected fruits from several plants of the four most common shrubs in the study area (Pacific blackberry, salmonberry, red Huckleberry and salal). As we had previously observed considerable slug damage to understory mushrooms at MKRF, we used another set of 35 slugs (15 natives and 20 aliens) to assess the rate of feeding on store-bought white button mushrooms (*Agaricus bisporus*) between banana and black slugs. We recorded the percent consumed of the total volume for fruits and mushrooms after 48 hours in each feeding trial (Strauss et al. 2009).

We used GLMs using a binominal distribution with a logit link function to compare the ratio of the volume of fruit and mushroom consumed over the total volume of the fruit and mushroom by native vs. alien slugs.

Field mesocosms

To measure the individual and combined impacts of native and alien slugs on forest understory plants, we established a field mesocosm experiment in a Hemlock-dominated overstory site at MKRF in July of 2012. Mesocosms consisted of 0.25 m² × 0.4 m enclosures cages (n = 37) in which we planted a standard density of common understory species (see below). We used native and alien slugs, as well as seedlings, collected from the study site. Cages were constructed using a pair of wooden cedar frames (0.5 m × 0.5 m × 0.2 m). We lined the bottom of one frame with window screen (to prevent slugs from escaping) and filled the cage 50% full of a standard store-bought top soil. We then transplanted a single seedling of each of the seven most common understory plants, including hemlock, cedar, Douglas fir, huckleberry, salal, *Rubus* spp., as well as a common sword fern frond. Finally, we filled 25% of the soil surfaces of the cage with moss (See S1 for photo of a mesocosm). Next, we covered the top of another wooden frame with window screen and stacked this frame on the one filled with native plants. We used duct tape to seal the seam between the two frames.

We distributed mesocosms haphazardly throughout the site in relatively flat areas free of woody shrubs. We then established experimental additions of slugs at low, medium and high slug densities, mimicking the densities of slugs observed in our pitfall trapping. Low-density treatments included 1 slug per mesocosm of either banana (n = 5) or black slugs (n = 5). Medium density treatments included 2 slugs of either banana (n = 3) or black (n = 3) slugs, as well as a mix containing one banana and one black slug (n = 3). Similarly, our high-density treatments contained 4 slugs of either banana (n = 3) or black slugs (n = 3), and a mix of two banana and two black slugs (n = 3). Finally, there were also control boxes containing no slugs (n = 9).

After 30 days, which was enough time for the slugs to consume food items in the mesocosms, we scored the slug damage on leaf. We visually assigned damage scores to

every seedling and fern frond. We scored each leaf to one of 11 damage categories based on percent leaf area removed (0, 1-5, 5-10, 10-20, 20-30...90-100%). The same observer (MARC) scored all damage to maintain consistency across samples. As there were no major differences in the response of individual plant species within plant functional groups, we combined data as tree seedlings, shrubs and ferns. Also, as we found no effect of slug density, we lumped together the different slug densities into four groups: alien ($n = 11$), native ($n = 11$), mixed ($n = 6$), and control ($n = 9$). Finally, we compared the effect of slug type (native vs. alien) and plant type on the percent leaf area removed (PLAR) using a two-way ANOVA. PLAR was log-transformed prior to analysis to improve normality and reduce heteroscedasticity. For clarity, we show the untransformed values in all figures. We used JMP Pro 10 (SAS Institute Inc., Cary, NC) for all analyses.

Metabolic rate

To compare metabolic rate between native and alien species, we collected ten banana slugs (Mean \pm SD; mass = 17.34 ± 5.80 g) and ten black slugs (mass = 8.67 ± 2.14 g) from the forest and transferred them to plastic containers (see details in Cafeteria experiment section above) in the laboratory. Two slugs of the same species were housed in each holding container and fed lettuce daily. Containers were also cleaned daily. All slugs were held at room temperature ($\sim 22^\circ\text{C}$) and at 12:12 L:D photoperiod in the lab for the entirety of the experiment (September 24th, 2012 to October 24th, 2012).

To obtain a proxy of standard metabolic rate (SMR), we monitored slug oxygen consumption following acute exposure to several temperatures. Oxygen consumption trials were conducted using air-filled 250 mL glass respirometers submerged in a temperature-controlled water bath. Oxygen consumption of both native and alien slugs was then measured at seven different temperatures: 5, 10, 15, 20, 25, 28, and 30°C . Temperatures were maintained using a Lauda RM6 compact thermostat (Lauda-Brinkmann, LP, Delran, NJ). Prior to testing, slugs were fasted for 24 hours, weighed and placed into respirometers moistened using sterile water to avoid desiccation. Slugs were placed in unsealed respirometers at the test temperature twelve hours prior to measurement of oxygen consumption, which was measured in sealed respirometers using Neofox oxygen probes (Ocean Optics, Dunedin, FL) with a sampling rate of once every 5 seconds. Probes were calibrated every day prior to data collection. Declines in oxygen levels were monitored for 40–120 minutes (depending on temperature), and the rate of oxygen consumption was determined from the linear portion of the trace. The final oxygen level at the end of the trial was never less than 90% of normal air. Following each trial, slugs were removed from the respirometers, re-weighed, returned to holding containers, and fed a standard lettuce diet. Slugs were fed for at least one 24-hour period before being fasted and tested at subsequent temperatures. Ten different slugs were tested per day (5 native, 5 alien), and the use of two oxygen probes allowed concurrent testing of two slugs (1 native and 1 alien). We used separate linear models with body mass as a covariate to determine whether alien and native slugs had different metabolic rates.

Results

Slug abundance

Overall, we found that black slugs were $\sim 9\times$ more abundant than banana slugs (GLM on Poisson distribution; $\chi^2 = 263.99$, $P < 0.0001$), and there was a significant interaction between slug type and forest type (Table 1 and Fig. 1). Alien slug abundance was higher in Douglas fir (mean: 25.5 slugs/site; SD: 14.25) and clear cuts (mean: 22.67 slugs/site; SD: 8.14) compared to cedar (mean: 7.75 slugs/site; SD: 7.27) and hemlock (mean: 7.75 slugs/site; SD: 6.95; $F_{3,14} = 3.63$, $P = 0.048$) overstories. Despite that the abundance of native slugs were $\sim 5\times$ and $\sim 1.5\times$ more abundant in cedar sites (mean: 2.50 slugs/site; SD: 1) and hemlock sites (mean: 2.25 slugs/site; SD: 1.50) than in Douglas fir sites (mean: 0.50 slugs/site; SD: 0.58) and clear cuts (mean: 1.67 slugs/site; SD: 1.53), we did not find a significant difference in the abundance of native slugs across the different habitat types (Fig. 1).

Cafeteria experiments

We found no difference between slug species in the amount of fruit consumed by the slugs in our cafeteria experiment (GLM on binomial proportions; $\chi^2 = 0.01$, $P = 0.9260$). We found that both native and alien slugs preferred Pacific blackberry, salmonberry and salal over the red Huckleberry (native: $F_{3,167} = 12.69$, $P < 0.0001$; alien: $F_{3,182} = 11.83$, $P < 0.0001$). On the other hand, alien slugs consumed $\sim 7\times$ the amount

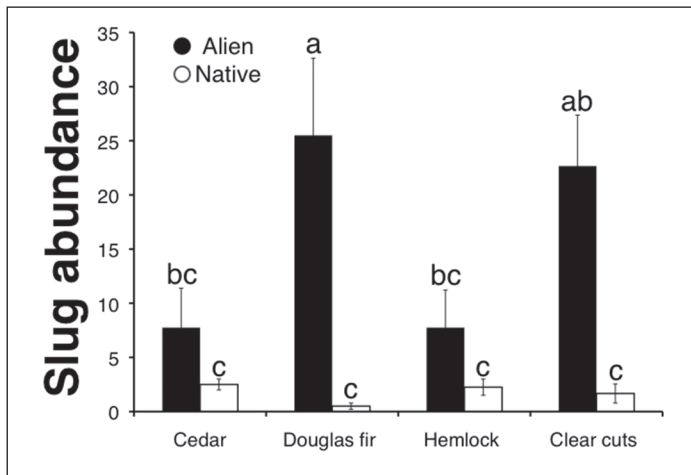


Figure 1. Alien black slugs were $50\times$ and $14\times$ more abundant than native banana slugs in Douglas fir and clear cuts respectively (mean \pm SE, letters indicate significant differences among treatments after separate Wilcoxon test corrected with Bonferroni corrections). Taking all the sites together the alien black slug was $\sim 9\times$ more abundant than the banana slug ($P < 0.0001$).

Table 1. Results from a GLM examining native and alien slug abundances across different forest types, including cedar, Douglas fir, hemlock, and clear cuts. Bold values are significant at $P < 0.05$.

	Source	df	χ^2	<i>P</i> -value
Slug abundance	Model	7	263.99	< 0.0001
	Forest type	3	2.18	0.5362
	Slugs (native vs. alien)	1	151.46	<0.0001
	Interaction	3	24.187	<0.0001

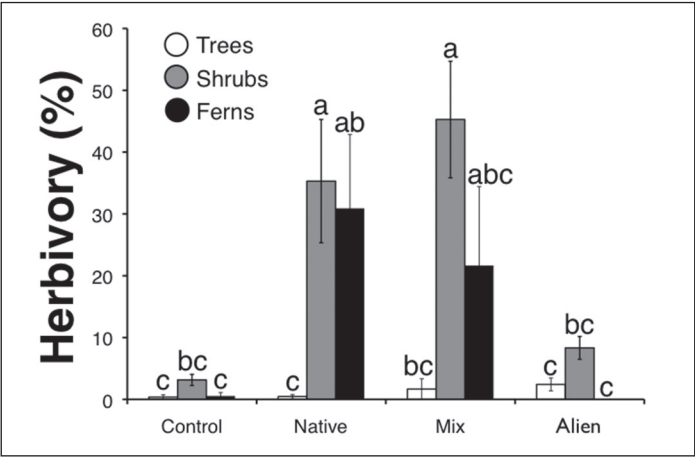


Figure 2. Herbivory was greater in banana slug and mixed enclosures than in alien and control enclosures ($P < 0.0001$), driving by greater preference of banana slugs for native shrubs and ferns compared to alien black slugs (mean \pm SE, letters indicate significant differences among treatments after separate Wilcoxon test corrected with Bonferroni corrections).

of white button mushrooms compared to native slugs (GLM on binomial proportions; $\chi^2 = 14.99$, $P < 0.0001$), which was consistent with our field observations of alien slugs commonly feeding on fungi fruiting bodies (i.e. mushrooms) in the forest (see S2 photo). We did not observe native slugs feeding on forest mushrooms.

Field mesocosms

We found that herbivory was ~6× higher in native slug and mixed slug enclosures compared to alien only and control enclosures (two-way ANOVA; $F_{11,99} = 10.08$, $P < 0.0001$; Table 2). Damage rates also varied between plant functional groups, with shrubs and ferns showing the highest levels of slug herbivory and tree seedlings the lowest (Fig. 2). There was no interaction between slug type and food type (Fig. 2), driven by native slugs consuming disproportionately more shrubs and ferns than alien slugs.

Table 2. Results from a two-way ANOVA comparing slug herbivore on different plant functional groups (trees, shrubs and ferns) in experimental mesocosms. Bold values are significant at $P < 0.05$.

	Source	df	<i>F</i>	<i>P</i> -value
Slug abundance	Model	11	10.08	< 0.0001
	Food type	2	29.56	< 0.0001
	Treatment	3	10.17	< 0.0001
	Interaction	6	3.74	0.0021

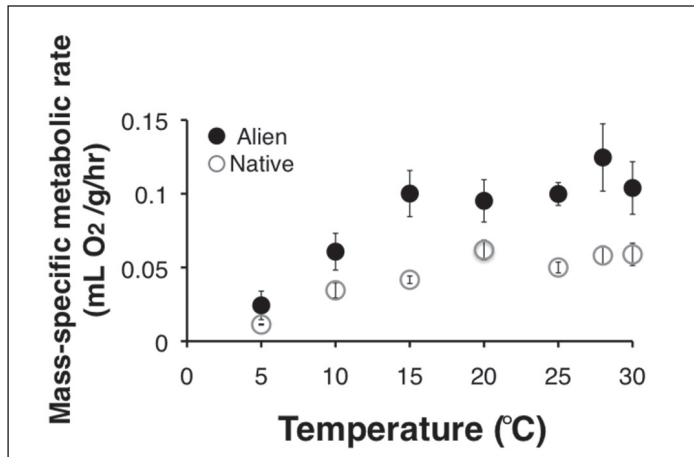


Figure 3. The effect of temperature on metabolic rate (MO_2) was similar for both native and alien slug species, but mass-specific metabolic rate was higher for the alien black slugs.

Metabolic rate

When we compared the effect of temperature on slug metabolic rate (MO_2), we found that the metabolic rates for both slug species have the same general qualitative pattern of response with change in temperature (Fig. 3). As expected based on thermodynamic considerations for ectothermic species, metabolic rate increased with temperature, but only up to approximately 15 °C, after which point it remained constant as temperature increased (Fig. 3). All slugs survived up to the highest temperature tested of 30 °C. Mass-specific metabolic rate was higher for the alien slugs than for the native slugs at all temperatures, but this difference resulted from the differences in body mass between the two groups, as the alien slugs were smaller. A linear model testing the effects of species, temperature, and body mass on MO_2 revealed a highly significant effect of body mass ($P < 0.0001$) and temperature ($P < 0.0001$) on MO_2 , but no significant effect of species ($P = 0.57$). In addition, there was a significant interaction between mass and species ($P = 0.002$).

Discussion

In this study we found that alien black slugs were more abundant and consumed a greater quantity of mushrooms than native banana slugs. The fact that alien slugs are much more prevalent compared to native slugs could be explained by two mechanisms. First, alien slugs could have a direct negative effect on native banana slugs. Alternatively, alien slugs might not reach high abundance where native slugs are performing well, in which case native banana slugs could have a direct negative effect on alien slugs in cedar and hemlock forests. Because we do not have data on native slug abundance prior to the invasion of alien slugs, we cannot verify or reject either of these two possible explanations without further study. Although some studies have shown that alien slugs can outcompete native slugs (Rollo 1983), other studies have shown no effect of the alien slugs on native slugs (Hamilton and Wellington 1981).

It is possible that the higher abundances of alien slugs found in Douglas fir forests might reflect their diet preferences for fungi, as indicated in our cafeteria experiments and field observations. While this was a simple assay using store-bought white button mushrooms, Douglas fir forests depend heavily on ectomycorrhizal fungi symbiosis and can have high abundances of mushrooms. For example, the presence of the fruiting bodies of ectomycorrhizal fungi have been shown to influence the abundance of small mammals in forest stands (Carey 1995, Maser et al. 1978). Given our observations of slug damage to mushrooms at our study site, we think that fungi represent an important food source for the alien slugs and warrant further study for understanding the impacts of slug invasions on native tree and fungal communities.

The cafeteria experiment also revealed that alien and native slugs have similar preferences for fruits, particularly berries that dominate the understory in coastal BC forests. We observed intact seeds passing through native and alien slug guts, suggesting the potential for seed dispersal. In fact, previous studies have found that banana slugs consume fruits of several shrubs in these forests, decreasing the survival of salmonberry, and increasing the germination rate of large-flower fairy-bells (*Prosartes smithii*, see Gervais et al. 1998 for a complete list of species). However, no study to our knowledge has been conducted comparing the roles of native and alien slugs as seed dispersers or seed predators in this system and these interactions warrant further study.

Our results from the mesocosms indicate that neither the native nor the alien slugs appear to consume conifer tree seedlings, which is a positive result for seedling plantations and forest management [but see, Cote et al. (2005), where slugs were the most common predators of seedlings of black spruce (*Picea mariana*)]. Moreover, alien slugs did not feed upon ferns and scarcely fed upon shrub seedlings in the mesocosm experiment (Fig. 2). Therefore, it is possible that slug invasion will have the greatest impact through the consumption of fruits of many of the understory berry and fungal species in the coastal forests of BC.

When we compared the effect of temperature on slug metabolic rate (MO_2), we found no differences in the metabolic rate for both slug species. Thus, the differences in

their diet are not based on different overall metabolic rates and both species are likely active at similar temperatures in the forest. The differences in the diet of these two species of slugs could be due to variation in the reproductive cycle or the anatomy of the slugs. First, the black slug completes its somatic growth and accumulates reproductive reserves within a single year (Rollo 1983a,b), while native banana slugs live for several years (Hamilton and Wellington 1981). Thus, alien slugs might have to feed more in order to complete its annual life cycle. Second, larger body sizes tend to have higher absorption rates and so, by being smaller, it is possible that black slugs would need to feed more than larger banana slugs (Hamilton and Wellington 1981). Moreover, our results indicate the potential for both native and alien slugs to act as seed or fungal spore dispersers or predators in the temperate rainforests of BC, which has been a poorly explored role of slugs. While further experimental work is required to reveal whether alien slugs are having a negative impact on banana slugs, our results suggest that alien slugs possess different traits in addition to shorter life cycle that make them functionally different from the native banana slugs. Consequently, the results of our study warrant further research, particularly if the alien black slugs are displacing native banana slugs, as these two species are the most conspicuous slug species in the forest ecosystems of the Pacific Northwest.

Acknowledgements

We thank Ionut Aron and MKRF for facilitating our field studies and Larissa Schaub for assistance carrying out the slug metabolic rate trials.

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Supplementary material 1

Mesocosm

Authors: Mariano A. Rodriguez-Cabal, Taylor C. Gibbons, Patricia M. Schulte, M. Noelia Barrios-Garcia, Gregory M. Crutsinger

Data type: JPEG Image.

Explanation note: Detail of the mesocosm

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Supplementary material 2

Alien black slug

Authors: Mariano A. Rodriguez-Cabal, Taylor C. Gibbons, Patricia M. Schulte, M. Noelia Barrios-Garcia, Gregory M. Crutsinger

Data type: JPEG Image.

Explanation note: Alien black slug eating a mushroom in the forest.

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Lantana camara L. (*sensu lato*): an enigmatic complex

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Academic editor: Ingolf Kühn | Received 4 July 2014 | Accepted 8 December 2014 | Published 14 April 2015

Citation: Goyal N, Sharma GP (2015) *Lantana camara* L. (*sensu lato*): an enigmatic complex. NeoBiota 25: 15–26. doi: 10.3897/neobiota.25.8205

Abstract

Lantana camara L., considered among the world's worst invaders is in identity crisis and contentiously referred as *Lantana camara* L. (*sensu lato*). Taxonomic ambiguity in *L. camara* L. (*sensu lato*), a species complex is one of the grim caveats behind incompetence of its management efforts. Recognizing the extent of variability within the complex, we aim to highlight the need to circumscribe its composition to bring effective management and control efforts into practice. There is a need for clear terminology to examine weedy, naturalized and/or invasive complex constituents that have been placed under the contentious umbrella of '*L. camara* L. (*sensu lato*)'. The time is ripe for invasion ecologists, cytogeneticists and conservationists to collaboratively focus on disentangling the complex and integrate their knowledge and expertise into management and control programs.

Keywords

Control, genetics, invasive, species complex, taxonomy

Introduction

Lantana camara L. is one of the world's worst ten invaders (Lowe et al. 2000). The copious invader has disseminated rapidly at temporal as well as spatial scale. This widespread invader inhabits a wide range of habitats adversely impacting native plant species diversity and ecosystem functioning (Gentle and Duggin 1997, Sharma et al. 2005, Love et al. 2009a, Sharma and Raghubanshi 2009). Despite profound environmental and economic threats posed by *L. camara* infestations in non-native range,

considerable success has not been achieved to effectively control its spread (Cilliers 1983, Bhagwat et al. 2012). Nevertheless, species invasiveness is too complex to be simply predicted and managed (Williamson 1996). However, we anticipate that the prime reason behind the failure to control invasive *Lantana* could be its existence as an unresolved species complex, *L. camara* L. (*sensu lato*). Inability to correctly identify the plant of interest in the complex has incapacitated control measures (Thomas and Ellison 1999, Day et al. 2003a, Urban et al. 2011).

Historically, taxonomy of the genus *Lantana* has been very complicated (Sanders 2006, Love et al. 2009b). Further, recognition of all weedy and/or invasive genets created due to endless episodes of horticultural improvement within the genus is extremely challenging. Over the last two to three decades, a few articles highlighted the seminal concept of addressing different weedy and/or invasive genets [cultivars, variants, sub-species, hybrids, varieties, forms or even allies (*if they exist*); hereafter, referred as complex constituents] as *L. camara* L. (*sensu lato*) (Stirton 1977, Stirton 1979, Sanders 2006, Sanders 2012; for details, see Urban et al. 2011). However, the importance of this concept has been realized in invasion ecology after decades of consistently delimiting all of the invasive complex constituents with the epithet *L. camara*.

Importantly, little information is available on taxonomic identity and genetic makeup of existent complex constituents and their populations. This further makes it difficult to surmise what facilitates their success as invaders. Considerably, complex constituents' invasive success might be ascribed to phenotypic and/or genotypic novelty created by rigorous hybridization events within the complex, their variable response to selection or their differential adaptive plasticity. However, in order to ascertain the key attribute(s) responsible for their invasive success, identification and differentiation of all possible invasive genets in the complex is fundamental. Detailed study of the complex constituents will offer opportunities to answer key questions about plant invasions. Moreover, it may be useful in making informed choices about monitoring their spread throughout the invaded range. The present synthesis will generate an impetus for comprehensive research efforts to study the remarkably diverse species complex in order to appraise control efforts.

Historical events leading to the species complex

Although specific origin of *L. camara* is unknown, some authors have suggested the species to be a native of South America or Mexico (Howard 1969, Spies and du Plessis 1987), while others suggested West Indies as the place of origin (Moldenke 1973, Palmer and Pullen 1995). Studies report that *Lantana* (including *L. camara*) was imported from America to Europe in mid-16th and 17th centuries for its horticultural value (Stirton 1977, Swarbrick et al. 1995). In Europe, the species underwent substantial horticultural breeding, creating hundreds of cultivars of mixed parentage from the introduced stock (Howard 1969). Subsequently, these cultivars traversed to America, Australia, India and Africa in the mid-19th century (Howard 1969, Stirton 1977, Swarbrick 1985, Morton

1994). With time, many cultivars escaped cultivation, spread beyond the ornamental confines of the garden and became weeds (Spies 1984, Swarbrick 1985, Palmer and Pullen 1995). Studies have identified hybridization to contribute substantially to invasiveness, weediness and/or range expansion (Brown and Marshall 1981, Ellstrand and Schierenbeck 2000, Hovick and Whitney 2014). Likewise, innumerable intentional as well as unintentional hybridization events in *Lantana* led to remarkable increase in its complexity. Anthropogenically-induced genetic diversity in the species complex indeed facilitated the species to invade heterogeneous habitats (Cilliers 1983, Bhagwat et al. 2012, Goncalves et al. 2014). The highly invasive species now exists in 60 countries and island groups of Asia, Africa and Australia (Cronk and Fuller 1995, Day et al. 2003b).

Enigma of the species complex

Extensive hybridization followed by polyploidy or polyploidization followed by hybridization events within and between wild, naturalized and cultivated taxa further enhanced complexity leading to the evolution of *L. camara* L. (*sensu lato*) (Sanders 1987, Sanders 2006). Wild complex constituents have also been reported to hybridize and genetically assimilate with the rare native counterparts, threatening the existence of rare genets (see Maschinski et al. 2010). These evolutionary processes have led to enormous phenotypic as well as genotypic variability, which complicates species delimitation in the complex. The ones growing in wild potentially differ morphologically, karyologically, physiologically and ecologically from those prized for their horticultural value, multicolored flowers, and ease of propagation (Spies 1984, Sanders 2006). Therefore, weedy, naturalized and/or invasive complex constituents, broadly referred as *L. camara* L. (*sensu lato*) merit a deliberate taxonomic delineation (Sanders 2006).

Complex constituents can be distinguished morphologically (flower size, shape and color; leaf size, hairiness and color; stem thorniness; height and branch architecture), physiologically (growth rates, toxicity to livestock) and, by their chromosome number, nuclear DNA content (Stirton 1979, Gujral and Vasudevan 1983, Scott et al. 1997) and ploidy level (Stirton 1977, Palmer and Pullen 1995). Studies have also reported leaf anatomical characteristics (Passos et al. 2009) and detailed chemical profiling of foliar chemical constituents (Love et al. 2009b, Sena et al. 2012) as useful markers for supporting species delimitation. However, obscure limits of natural variation hamper workers in the field to effectively classify and disentangle complex constituents. The disputed limits of *L. camara* also complicate identification of the genotypes that have naturalized and are proliferating in the non-native range.

Understanding the species complex

Unlocking diversity in the complex is considered a formidable taxonomic problem (Khoshoo and Mahal 1961, Howard 1969, Moldenke 1971, Spies 1984, Sanders

2006). The complex with a broad spectrum of variability has no record of parental species after 1492 (Stirton 1977). It is highly difficult to deduce putative parents of each constituent in wild as they interbreed freely leading to immense variation in the gene pool (Binggeli 2003, Spies 1984, Urban et al. 2011). Further, ongoing hybridization events in the cryptic complex have blurred taxonomic distinctions of complex constituents and reduced classification accuracy (Sanders 2006, Maschinski et al. 2010). Although the taxonomically uncertain complex has not been subjected to considerable progress till date; a few studies have attempted to reasonably explore the composition and nomenclature to classify the genus *Lantana* through well-devised keys (Munir 1996, Rajendran and Daniel 2002, Méndez Santos 2002, Sanders 2006, Sanders 2012).

Cytological studies on different populations of *Lantana* have reported basic chromosome numbers to be 11 & 12 (Henderson 1969, Sinha and Sharma 1984) and 8 & 11 (Moldenke 1983). They have also pointed out the existence of polyploid series in the genus based on reported base numbers. Highest chromosome number was reported as $2n = 72$ (Natarajan and Ahuja 1957), and $2n = 66$ (Bir and Chatha 1983), while $2n = 22$ was recorded to be the lowest (Sen and Sahni 1955, Sanders 1987). Basic chromosome numbers of 11 & 12 have been recorded, with ploidy levels ranging from diploid to hexaploid in *L. camara* (Tjio 1948, Spies 1984, Ojha and Dayal 1992, Munir 1996, Brandao et al. 2007). Frequent hybridizations between different ploidy levels have also been reported (Spies 1984). Existence of multiple polyploidization pathways has been considered to contribute towards enormous complexity in *L. camara* (Czarnecki II and Deng 2009).

Rapid adaptive evolution and genetic change have been proposed to contribute significantly to the success of invasive species in the introduced range (Prentis et al. 2008, Prentis and Pavasovic 2013). Studies have revealed significant information regarding genetic variation, population differentiation, and introduction history of a few invasive species using molecular markers (Chun et al. 2010, Thompson et al. 2012, Vardien et al. 2013). Few studies have also attempted to explore range expansion of *L. camara* in different countries (Vardien et al. 2012, Ray and Quader 2014). Microsatellite markers developed for *L. camara* have also been successfully used in assessment of genetic variation and population structure in India, broadening understanding on dynamics of its introduction, range expansion and gene flow (Ray and Quader 2014, Ray and Ray 2014). Ray and Quader (2014) identified that the present diversity of *L. camara* in India is an output of multiple introduction episodes followed by gradual spatial expansion with the recurrent gene flow. Recently, Ray and Ray (2014) studied genetic variation in *L. camara* in India and synthesized that the species consists of two genetic clusters, representing emerging ecotypes across space that could be differentially adapted to local habitat conditions. Broadly, these studies have revealed high genetic diversity in *L. camara* and have tried to elucidate past dispersal patterns (Vardien et al. 2013, Ray and Quader 2014, Ray and Ray 2014). Further, these markers can be useful in addressing several questions about breeding system, pollination and dispersal of the species (Ray et al. 2012). However,

to improve our understanding of the range expansion of the complex, there is a need for further research at a global scale that examines genetic and genomic attributes of the complex constituents. It is highly essential to understand the genetic system of a taxon as it affects the nature and extent of variability, evolutionary processes and pathways which may further affect invasiveness.

Unresolved species complex: an impediment to management efforts

Interestingly, a large proportion of invasive alien plants are those that were introduced as ornamentals (Mack and Lonsdale 2001, Foxcroft et al. 2008). In general, ornamental plants selected for introduction pose a high invasion risk as they possess traits such as high fruit/seed production, high growth rate, and tolerance to a wide range of environmental conditions (Anderson et al. 2006).

Highly variable *L. camara* is one amongst those introduced ornamental species that has a wide scale of distribution. In spite of longer residence time, there is a dearth of scientific studies integrating its history, spread, ecological impact, evolution, and management. Thus, attempts to control the invader using mechanical, chemical and biological means have met with limited success (Morton 1994, Thomas and Ellison 2000, Day et al. 2003a). Additionally, this can also be attributed to a gamut of complex constituents that differ in their distribution, habitat preferences, weediness, morphology, chemical constituents, toxicity to livestock, susceptibility to herbicide treatment, and susceptibility to bio-control agents (Smith and Smith 1982, Cilliers and Naser 1991). Further, success of bio-control agents employed in controlling *L. camara* (including all weedy and/or invasive complex constituents) may vary from one location to another owing to differential feeding habits of the bio-control agents, their host-specificity, climatic suitability, and plant-insect interactions (Broughton 2000, Zalucki et al. 2007). Biological control measures are principally constrained by our confounding understanding of the broad spectrum of phenotypic and genotypic variability present within the complex. However, genetic analysis of the complex can aid in identification of potential control agents to be specifically targeted (Scott et al. 2002).

Future directions

Under the current scenario of genetic diversity and associated taxonomic ambiguity, distinction of genets in *L. camara* L. (*sensu lato*) is highly dubious. However, a few studies erroneously address invasive *Lantana* with ambiguous identity as *Lantana camara* L. or *Lantana camara* L. (*sensu stricto*), which is extremely misleading with the current understanding of the complex. Merely considering *L. camara*, representative of all troublesome weedy genets in the whole complex, will neither ensure understanding all of the myriad invasive traits, nor would it serve to appropriately answer

management questions. Hence, studies pertaining to invasive *Lantana* should address the individuals in the wild with the epithet '*L. camara* L. (*sensu lato*)' (Stirton 1977, Naser and Cilliers 1990, Munir 1996, Baars and Naser 1999, Day and Zalucki 2009). Referral of the invasive complex constituents broadly as *L. camara* L. (*sensu lato*) is considered correct under both the International Code of Botanical Nomenclature and the International Code of Nomenclature for Cultivated Plants (see Urban et al. 2011).

To circumscribe the complex constituents, documentation of appropriate suite of distinguishing characters of complex constituents might facilitate delineation of the considerable variation existing in the complex. A consistent terminology based on morphology, cytology, and genetic attributes using advanced molecular techniques such as DNA-based molecular marker techniques, *viz.* random amplified polymorphism DNA (RAPD), inter simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), quantitative trait locus (QTL) mapping, etc. can be devised to explore the genetic diversity of the complex constituents. Integrating the knowledge of plant morphology and chromosome number can also aid in species delimitation, as complex constituents have been reported to behold varying chromosome complements. Attempts to investigate total spectrum of variation using DNA C-values by flow cytometry can be extremely helpful to unravel the diversity in the complex (Suda et al. 2014). Though highly desirable, yet the extremely difficult task of ascertaining absolute taxonomic status to each of the complex constituents can be resolved by estimation of their genome sizes. Variation in chromosome numbers in genus *Lantana* encourages the use of genome size as a species-specific marker. Genome size has been well-applied to resolve notable species complexes such as *Reynoutria* (Mandák et al. 2003); *Knaulia arvensis* (Kolář et al. 2009); *Dryopteris carthusiana* (Ekrt et al. 2010); *Callitriche* (Pranč et al. 2014) and, identification of invasive alien taxa (see Suda et al. 2010). Further, documentation of population cytotype structure of the invasive genets and their geographical distribution is central to monitor complex constituents' invasion potential.

High diversity in the complex and continuing hybridization events may potentially broaden its ecological tolerance in climatically suitable as well as unsuitable areas (Goncalves et al. 2014). Furthermore, realizing remarkable spread and better performance of the invasive genets in warmer areas, it is highly probable that invasive *Lantana* will increase its expanse noticeably in future climate change scenarios (Zhang et al. 2014). Control of the invader would be quite challenging in future scenarios of global change. Lack of knowledge about actual genetic diversity in the complex will further undermine all efforts to regulate species' invasion dynamics.

Concluding remarks

The study warrants that there is an urgent need to resolve the species complex to ensure concerted management and timely control over its proliferation. In a nutshell, future of efforts to control invasive *Lantana* lies with resolution of the species complex. There is an urgent need to disentangle the complex to decipher the niche adaptation and

range expansive modifications that distinct complex constituents underwent over the evolutionary timeframe. Using insights, we can build-up and enhance our understanding of different facets of *L. camara* L. (*sensu lato*) invasion in entirety.

Acknowledgements

NG acknowledges Senior Research Fellowship (SRF) support from University Grants Commission, India. GPS acknowledges funding support from University of Delhi, India and Department of Science and Technology, India. Authors are grateful to the anonymous referee for the constructive criticism.

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Factors influencing the local scale colonisation and change in density of a widespread invasive plant species, *Lantana camara*, in South India

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Academic editor: M. van Kleunen | Received 29 July 2014 | Accepted 27 January 2015 | Published 14 April 2015

Citation: Sundaram B, Hiremath AJ, Krishnaswamy J (2015) Factors influencing the local scale colonisation and change in density of a widespread invasive plant species, *Lantana camara*, in South India. NeoBiota 25: 27–46. doi: 10.3897/neobiota.25.8354

Abstract

Identifying factors that underlie invasive species colonisation and change in density could provide valuable insights into the mechanisms of biological invasions and for invasive species management. We examined a suite of factors potentially influencing the landscape-level invasion of *Lantana camara* L., one of the most ubiquitous invasive species in South Asia. These factors included disturbance factors like forest fires, historical habitat modification, and edge effects, in addition to factors like propagule pressure and habitat suitability. We examined the relative importance of these factors on the colonisation and change in density of *L. camara* in the Biligiri Rangaswamy Temple Tiger Reserve, Western Ghats, India. We used extensive (1997–2008) datasets tracking the presence and abundance of *L. camara* and combined these with corresponding data on disturbances, propagule pressure, and habitat suitability. We used an information-theoretic model selection approach to determine the relative importance of each factor on the colonisation and change in density of *L. camara*. Colonisation was mainly a function of proximity to already established populations (i.e. propagule pressure), whereas increase in *L. camara* density appeared to be constrained by high fire frequency. Research and management efforts need to recognize the multi-dimensional nature of mechanisms underlying *L. camara*'s success during different invasion phases when strategizing interventions to mitigate its effects.

Keywords

Tropical dry forest, disturbance, propagule pressure, forest fire

Introduction

The likelihood of an introduced species becoming invasive is determined by the interplay of the invader's characteristics (e.g. its high propagule output; Sakai et al. 2001), the abiotic environment (particularly disturbance; Shea and Chesson 2002), and biotic interactions within novel environments (e.g. enemy-release; Keane and Crawley (2002)). The disturbance regime prevalent in an area plays a pivotal role in influencing invasive species success (Davis et al. 2000). Habitats that are subject to natural or anthropogenic disturbances could be particularly vulnerable to invasion (Jauni et al. 2014). In addition to ongoing disturbances, disturbance legacies (e.g., historical clear-cutting), could also make habitats vulnerable to invasion (Seabloom et al. 2003). Lastly, feedback loops between periodic disturbances (like forest fires) and invasive species dominance have been observed in some systems (e.g. the grass-fire cycle; D'Antonio and Vitousek 1992), indicating the pivotal role played by disturbance in biological invasions.

Propagule pressure has also been found to act as an important driver in the invasion process (Rouget and Richardson 2003, von Holle and Simberloff 2005, Lockwood et al. 2005). In an experimental study using forest understory plants, von Holle and Simberloff (2005) found that successful invasions were contingent upon the number of propagules arriving *in situ*, when compared to other factors such as resident diversity and the flooding regime. Propagule pressure, in turn, is dependent upon life history characteristics such as time taken to reach reproductive maturity and dispersal mode – rapidly maturing species with abiotically dispersed seeds tend to be more successful compared to slow-maturing species with biotically dispersed seeds (Daehler 1998).

Both colonisation (i.e. local arrival in a part of the landscape where it was earlier absent), and change in density (i.e. changes in the abundance over time) contributes to invasive plant spread. Change in density influences the probability of maintaining populations in colonised sites and the quantum of propagules released from colonised sites. However, factors that influence colonisation may not necessarily influence density. At the colonisation stage, factors such as proximity to propagule sources may come into play more than factors such as habitat heterogeneity (Rouget and Richardson 2003). Although propagule pressure is largely a function of distance to invasion foci (Rouget and Richardson 2003), proximity to sites of historical or contemporary disturbance may directly determine proximity to propagules. It is therefore necessary to quantify the effects of these factors on invasive plant colonisation, individually, and in tandem.

Change in density, on the other hand, may be determined by the frequency of forest fires or other landscape-level disturbances (Jauni et al. 2014) rather than propagule pressure alone. Although von Holle and Simberloff (2005) experimentally established the primacy of propagule pressure as a determinant of invasions relative to other factors like resident species density and abiotic disturbance (flooding), other studies indicate that disturbance-triggered regeneration favours invasive species over native species (Galatowitsch and Richardson 2005). Given uniform propagule pressure, it could be possible that exploiting windows of opportunity provided by disturbance are key to invasive species success. Although the role of disturbance in the invasion process has

been described in many studies, the specific role of forest fire as a determinant of the spread of invasive species is unclear.

Lantana camara is one of the most globally ubiquitous invasive species (Cronk and Fuller 1995, Day et al. 2003, Vardien et al. 2012). Reports thus far suggest that in some contexts an increase in fire frequency could increase *L. camara* density due to its capacity to readily resprout in response to fire (Hiremath and Sundaram 2005), while in some others, an increase in fire frequency could depress *L. camara* density, possibly by depleting the soil seed bank (Sundaram et al. 2012, Debuse and Lewis 2014).

L. camara was introduced to India a little over two centuries ago, and is today one of the most widespread invasive plant species in the country (Kannan et al. 2013). Based on herbarium records, Kannan et al. (2013) mapped the introduction and spread of *L. camara* across India in the 19th century. Their results show that the spread of *L. camara* in India started in the early 1800s, and accelerated with the development of travel networks and the expansion of colonial forest management thereafter. Yet, while we now have information on temporal and spatial patterns of *L. camara* spread at a national and regional scale, we have so far lacked information on the mechanisms underlying its local scale colonisation and increase in abundance.

We examined factors underpinning the colonisation and change in density of *L. camara* in the Biligiri Rangaswamy Temple Tiger Reserve, a seasonally dry tropical forest landscape in the Western Ghats biodiversity hotspot, India. Rapid *L. camara* invasion has occurred here over the past decade (Sundaram and Hiremath 2012). Given the gaps in our understanding of the *L. camara* invasion process, and the apparent multivariate nature of the factors that influence *L. camara* invasion—and species invasions in general (Gurevitch et al. 2011)—we used an information-theoretic, model-selection approach (Burnham and Anderson 2002) to determine the relative importance of propagule pressure, fire frequency, landscape history, habitat suitability, and contemporary disturbance on the colonisation and change in density of *L. camara*. We expected that colonisation would be driven by propagule pressure while *L. camara* change in density would be driven by disturbance and forest fires.

Methods

Lantana camara L.

Lantana camara (Verbenaceae) is a straggling shrub native to South and Central America. Kannan et al. (2013) document records of multiple introductions of *L. camara* into India beginning from the early 1800s. It was introduced as a garden ornamental because of its attractive – and profuse – flowers, and was also extensively cultivated as a hedge plant. Its abundant sugar rich fruits attract a variety of frugivores, and *L. camara* is dispersed by birds (Bhatt and Kumar 2001) and small mammals (B. Sundaram personal observation). *Lantana camara* is also able to propagate vegetatively by resprouting from rootstock (Day et al. 2003).

Lantana camara started to be mentioned in the literature as invasive about a hundred years after its introduction to India (Tireman 1918, Iyengar 1933). Today, *L. camara* is common in tropical dry forests, slash-and-burn fallows, and pasture-lands all over India (Sharma et al. 2005).

Study area

The Biligiri Rangaswamy Temple Tiger Reserve (hereafter, BRT) in Karnataka, India, where this study was conducted, is part of the Western Ghats biodiversity hotspot (Das et al. 2006). The sanctuary is 540 km² in area, and is located between 77°00'–77°16'E, and 11°47'–12°09'N. The terrain is undulating, and elevation ranges from about 600 m in the foothills to about 1800 m in the upper reaches. Annual rainfall in the study area varies along this altitudinal gradient, ranging from ca 900 mm at low elevations to ca 1750 mm at higher elevations. The mean annual temperature is 25.3 °C, but varies with elevation. Winter temperatures drop to a mean minimum of 11 °C at higher elevations, while summer temperatures soar to a mean maximum of 42 °C in the foothills (Murali et al. 1998). Soils are moderately shallow, deep gravelly to sandy clay loams, with parent material classified as inceptisols consisting of granitic gneiss and charnockites (Anon. 1996). The area was declared a Wildlife Sanctuary in 1973 (Barve et al. 2005) and a Tiger Reserve in 2010.

Our study site is an ideal system to examine the mechanisms underlying *L. camara* invasion for two significant reasons. First, spatially explicit data on the presence and abundance of *L. camara* are available for 1997 (Murali and Setty 2001) and 2008 (Sundaram and Hiremath 2012), enabling us to examine the change in *L. camara* distribution and abundance over an 11-year period. Second, we have information on potential factors that may influence colonisation and change in density, which corresponds temporally and spatially with the data on change in *L. camara* distribution and abundance. The study area has a long history of human habitat modification and use. Forestry activities began during the 1930s (Ranganathan 1934) with the establishment of forest blocks to harvest sandalwood (*Santalum album*), bamboos (both *Bambusa arundanacea* and *Dendrocalamus strictus*), and timber (via selection- and clear-felling). In addition to disturbance generated by forestry activities, shifting agriculture was practiced by an indigenous tribe, the Soliga, in several sites across the BRT landscape over centuries, and this entailed the clearing and burning of understory vegetation prior to planting food crops (Madegowda 2009). Early dry-season fires, initiated by the Soliga for landscape management to clear the understory, enable the collection of forest products, and maintain trails, are characteristic of the BRT landscape (Sundaram et al. 2012). Subsequent to BRT being declared a Wildlife Sanctuary, logging (both clear- and selection-felling) and plantation activities were suspended, and most *podus* (Soliga settlements) within the area were relocated to the park's periphery (Fig. 1).

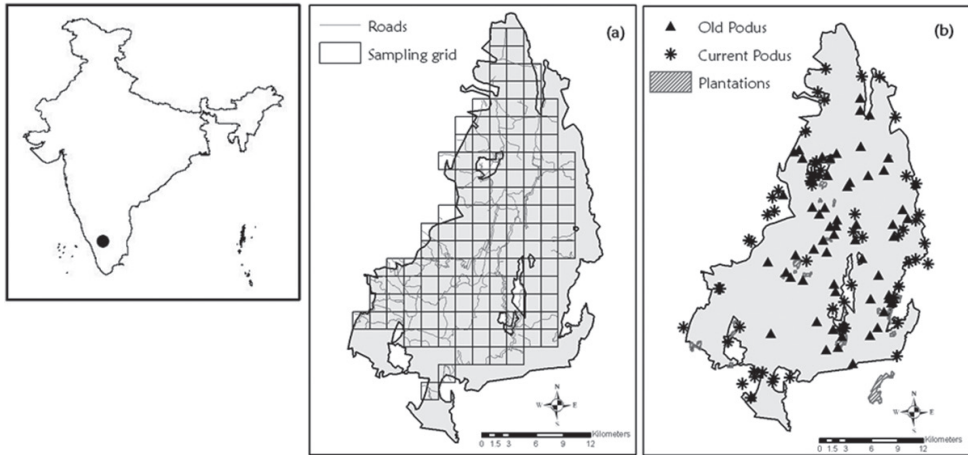


Figure 1. Map of the Biligiri Rangaswamy Temple Tiger Reserve (BRT) showing sampling grids and roads (a), old and current podus (Soliga settlements), and areas of historical plantation activity (b). Inset map of India shows location of BRT.

There are several forest types in BRT, of which seasonally dry forests constitute approximately 90% of the study area (Krishnaswamy et al. 2009) and include scrub-savanna, dry deciduous, and moist deciduous forests. These forests are extensively invaded by *L. camara* (Sundaram and Hiremath 2012).

***Lantana camara* colonisation and change in density**

Information on *L. camara* distribution in BRT from 1997 came from Murali and Setty (2001), who overlaid a 2×2 km grid over the entire study area and established plots at the center of each of the 134 grid cells during January-April 1997; plots were 80×5 m in size, with the long axis of plots oriented north-south. They counted and identified all woody stems >1 cm diameter at breast height (dbh) in each plot. Between August 2007 and January 2008, we used the same plot locations and field protocol used by Murali and Setty (2001) to enable assessment of colonisation and change in density since 1997. Twelve of the original 134 plots were found to occur in habitation or plantation during our survey, presumably as a result of a change in land-use at these locations between 1997 and 2008, and data from these plots were excluded.

Spatially explicit data on *L. camara* distribution from 1997 to 2008 enabled us to arrive at our response variables, colonisation and change in density. For examining colonisation between 1997 and 2008, we used the subset of plots that were uninvaded by *L. camara* in 1997 ($n = 71$). *Lantana camara* change in density between 1997 and 2008 was examined by computing the change in *L. camara* stem density from 1997 to 2008 (stem density in 2008/stem density in 1997). For this we used the subset of plots

that were already invaded by *L. camara* in 1997 ($n = 51$). Although hypothetically plots may already have attained maximum *L. camara* density in 1997, results from Murali and Setty (2001) suggest low density. The mean density of lantana increased more than three-fold between 1997 and 2008 (Sundaram and Hiremath 2012).

Predictor variables and model parameters

(a) *Lantana camara* neighbourhood index

We developed a *L. camara* neighbourhood index (LNI) assuming that presence of *L. camara* in an adjoining grid cell would contribute to propagule pressure. To calculate LNI in 1997, we used data on *L. camara* presence/absence from Murali and Setty (2001) and scored each grid cell in the study area as 0 (*L. camara* absent) or 1 (*L. camara* present). For each grid cell, the eight adjacent cells were listed and *L. camara* presence/absence scores were then averaged to generate a value of LNI and used as a proxy for propagule pressure exerted on a particular cell. The procedure we used to generate the LNI is admittedly a coarse-scale measure, since we are not taking into account within-grid heterogeneity, but are using only plot data to infer *L. camara* presence/absence across the entire grid cell. It could also be argued that this neighbourhood index is a proxy not just for propagule pressure, but for proximity to habitat suitable for *L. camara*. However, we have assumed that the effect of propagule pressure overrides the effect of habitat suitability in this case, based on findings from previous studies. For example, in their study of the distribution and cover of 3 invasive tree species in South Africa's Agulhas Plains, Rouget and Richardson (2003) found that the intensity of propagule pressure, approximated from the distance to putative invasion foci was a better predictor of invasive species cover than other environmental factors. They also found that population development (in terms of both colonisation and change in density) was driven primarily by propagule pressure; environmental variables came into play only later in the invasion process. That propagule pressure could overwhelm biotic resistance to invasion, and potentially override the effects of disturbance is also suggested by D'Antonio et al. (2001) in the context of plant invasions in California. This occurs because biotic resistance (and disturbance) is variable in space and time, creating windows of opportunity that could be exploited by propagules, if present.

(b) Degree of deciduousness of the canopy

Published information (Day et al. 2003, Sharma et al. 2005, Gentle and Duggin 2006) and observations from our study area suggest that *L. camara* is present in high densities in dry- and moist-deciduous environments compared to wet evergreen environments (Sundaram and Hiremath 2012). Thus, habitat suitability for *L. camara* may be related to canopy deciduousness. An index of '*eco-climatic distance*', a proxy for the degree of

deciduousness (DOD) and green cover, developed for the Western Ghats and BRT by Krishnaswamy et al. (2009), was used because it takes canopy biomass and its intra-annual variability into account. The DOD is a Mahalanobis distance of a pixel to a reference class (in this case, evergreen forest), that uses remotely-sensed, multi-date Normalized Difference Vegetation Index (NDVI) to arrive at a degree of deciduousness for each pixel (Krishnaswamy et al. 2009). Each pixel in the Krishnaswamy et al. (2009) study was 23.5 m × 23.5 m, while the plot size used in this study is 80 m × 5 m. To account for the difference in size between remotely-sensed DOD and plots from which ground data were gathered, values of DOD were averaged for all pixels encompassed within each plot.

Satellite images that were used for the derivation of degree of deciduousness by Krishnaswamy et al. (2009) were from 1998–1999. The density of *L. camara* in BRT was negligible during this period, as we know from the survey carried out just one year prior to this (Murali and Setty 2001). Data from Murali and Setty (2001) indicated that only 2.2% of plots invaded in 1997 had more than 50 lantana stems/plot (Sundaram and Hiremath 2012).

(c) Disturbance

Historical disturbance: Field observations from BRT indicated that a large proportion of historical plantations and agricultural sites are heavily invaded by *L. camara* (B. Sundaram *personal observation*). It is possible that these plantations and habitations were the original source locations from which *L. camara* spread. Historically, plantations of silver oak (*Grevillea* spp.) and teak (*Tectona grandis*) were established in multiple locations. Information about the locations of historical clear- and selection-felling sites was obtained from the field (using a hand-held GPS unit) and from Karnataka Forest Department records (Ranganathan 1934) for the study area. Additionally, locations of historical Soliga habitation sites were obtained from an existing study (Madegowda 2009). The distance from the plot center to the edge of historical plantation and agricultural sites, and from the plot center to the edge of historical Soliga habitation were computed. The lesser of these distances yielded the minimum proximity to historical disturbance (HD).

Contemporary disturbances: Several studies indicate that edge effects (estimated through proximity to habitation, and to roads and streams) may play an important role in *L. camara* invasion (Day et al. 2003, Bradley and Mustard 2006, Raizada et al. 2008). All roads within the study area were mapped using a GPS set to record points every 10 m, while streams were digitized from Survey of India topographical sheets. The minimum perpendicular distance from the plot centre to either roads or streams was calculated to yield a variable called EDGE. The locations of current habitation were recorded in the field using a GPS, digitized, and minimum perpendicular distance in metres between the center of each plot and the habitation boundary was calculated using MapInfo. This yielded a second variable called distance to contemporary disturbance (CD).

Fire frequency (FF): Burnt areas were mapped each year from 1997–2002 (R. Siddappa Setty, unpublished data), and 2004–2007 (this study), yielding fire maps for 10 of the 11 years over which change in *L. camara* distribution has been assessed. Each year during April–May, all motorable roads in BRT were traversed. Visibly burnt areas were marked on a topographical sheet (scale 1:50000, or 1 cm = 500 m). Additional burnt areas that were not visible from the roads were mapped from vantage points within BRT. At least 17 locations across the study area were consistently used as vantage points annually. The topographical sheets on which fires were mapped were scanned and burnt areas were digitized using MapInfo. Based on fire maps from 1997–2002 and 2004–2007 fire frequency was calculated for each grid cell as the total number of times the cell burned between 1997 and 2007. In case the area in a grid cell was incompletely burnt, the grid cell was scored as burnt only if the grid center (where plots were located) was burnt.

After deriving all predictors, we determined the level of correlation (Pearson's r) among predictor variables. We found a significant positive correlation ($r = 0.61$) between the minimum distance to historical plantation sites and minimum distance to historical habitation sites. Since our final predictor variable for historical disturbance (HD) was derived from the lesser of the two distances to historical plantation sites or historical habitation sites, this correlation would not affect our model selection exercise. None of the other predictors (or their components) were correlated.

Statistical analyses

Analyses were performed separately on data subsets corresponding to colonisation and change in density. An information-theoretic, model-selection approach (Burnham and Anderson 2002) was adopted to evaluate suitability of various predictors to describe the two response variables—colonisation and change in density. Rather than employing conventional hypothesis testing approaches, we used a model-selection approach in order to identify the relative importance of several predictors of *L. camara* invasion, both individually and synergistically.

After response and predictor variables were derived, candidate sets of models (global model with all predictors, single predictor models, and models containing predictor pairs) were developed *a priori* to examine factors driving colonisation and change in density. For both candidate sets, the explanatory variables used were identical, and in each candidate set of models, all predictor variables were used an equal number of times. Having a balanced model set was vital for the purposes of calculating the importance of each predictor variable individually (Burnham and Anderson 2002, Doherty et al. 2012). The predictor variables used in both candidate sets of models were (a) fire frequency (FF), (b) degree of deciduousness (DOD), (c) minimum distance to historical disturbance (either plantations or habitation; HD), (d) minimum distance to contemporary disturbance (existing habitation; CD), (e) minimum distance to edge (either roads or streams; EDGE), and lastly, (f) *L. camara* neighbourhood index (LNI).

Both candidate sets (one each for colonisation and change in density) contained a global model that included all predictors. The global models of colonisation and change in density included only two-way interactions (e.g., FF:EDGE) between predictors. Three-, four- and five-way interactions could not be included on account of small sample sizes. After defining the global model in each candidate set, a subset of separate single-predictor models was then added (a total of six models, one for each predictor). Finally, a subset of separate two-way additive models containing all unique combinations of predictor variables was added (a total of 15 models). Thus each candidate set had a total of 22 models (1 global model + 6 single predictor models + 15 two-way additive models).

A generalized linear model (GLM) with binomial errors and a logit link was used to model colonisation. For modeling *L. camara* change in density, a GLM with Gaussian errors and a log link was used. To account for instances where the density of *L. camara* in 2008 was 0 (a total of seven cases), we added a miniscule number (0.001), so that the log value of 2008 density/1997 density could be calculated.

For both candidate sets of models, a set of background tests were conducted before progressing to the model-selection stage (Burnham and Anderson 2002). The fit of the global model (% variance explained) was first examined. The data were then checked for overdispersion (i.e., if the sampling variance exceeded the theoretical (model-based) variance in the global model; Burnham and Anderson 2002). There was no evidence of overdispersion in either colonisation ($\hat{c} = 0.12$) or change in density data ($\hat{c} = 0.43$). After checking for overdispersion, Akaike's Information Criterion, corrected for small sample sizes (AIC_c ; Burnham and Anderson 2002) was calculated for each model. By including a bias correction term for complexity and small sample sizes, AIC_c estimates provide a maximum-likelihood based parsimonious measure of model fit. For each model in a candidate set, Δ_i (the value of AIC_c in the i^{th} model – minimum value of AIC_c occurring within the candidate model set) was calculated (Burnham and Anderson 2002). Based on Δ_i values, Akaike weights (relative likelihood of a focal model versus all hypothesized models) were calculated (Burnham and Anderson 2002). Lastly, by summing the Akaike weights for all models where a particular predictor appears, the "weight of evidence of support" (e.g., Marchetti et al. 2004) for each predictor was calculated across all models within a candidate set. Data analysis was conducted using R version 2.9.0. (R Development Core Team 2009).

Results

Lantana camara colonisation

The probability of colonisation increased with an increase in the *L. camara* neighbourhood index around each plot in 1997 (Fig. 2a). The model that used only the *L. camara* neighbourhood index as a predictor of colonisation had the lowest AIC_c value and the highest Akaike weight when compared to all other models (Table 1). Although the *L.*

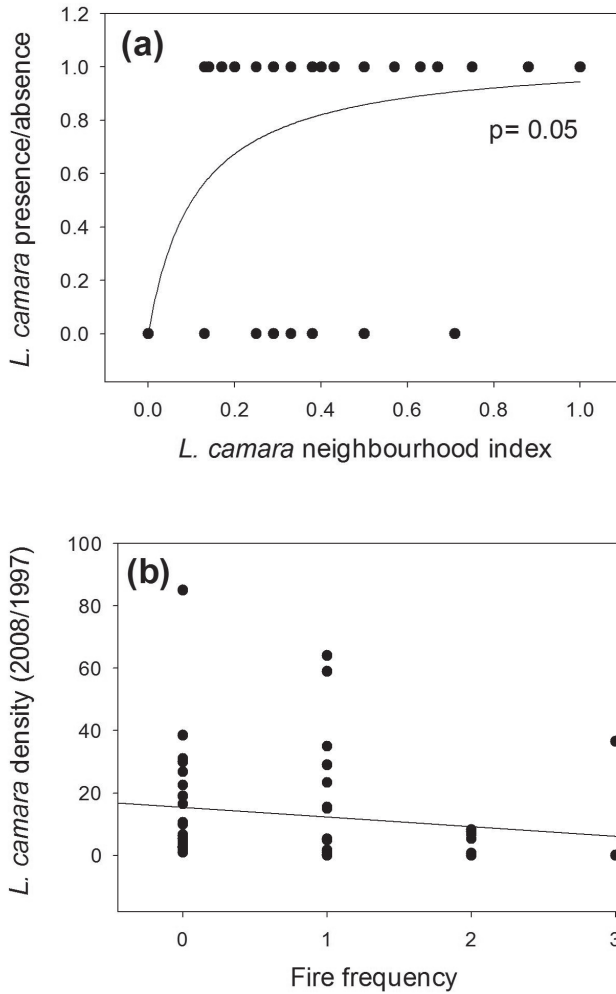


Figure 2. Variables that best explain *L. camara* colonisation (a), and change in *L. camara* density (b). P-values are taken from single-predictor GLM analyses.

camara neighbourhood index explained only 6.2% of the deviance from the intercept-only model for colonisation data, parameter averaging indicated that the *L. camara* neighbourhood index had the highest evidence of support as a predictor of colonisation when compared to all other covariates (Table 3). The value of the *L. camara* neighbourhood index around 71 plots uninvaded in 1997 varied from 0 (*L. camara* free neighbourhood) to 1 (completely *L. camara* invaded neighbourhood), with an average the *L. camara* neighbourhood index of 0.38 ± 0.021 . By 2008, a large majority of these plots ($54/71 = 76\%$) were invaded by *L. camara*.

Other disturbance factors that affect the study area, such as fire frequency and proximity to edge, did not emerge as being important for explaining *L. camara*

Table 1. Models to explain *Lantana camara* colonisation with their corresponding AIC_c (corrected AIC), Δ_i (value of AIC_c in the i^{th} model – minimum value of AIC_c), Akaike weights and percent deviance explained. The single-predictor model explaining the maximum deviance is in bold typeface.

Model	AIC_c	Δ_i	Akaike weights	% deviance explained
<i>L. camara</i> colonisation (n= 71)				
Global	116.05	38.27	0.000	51.12
Fire frequency (FF)	81.70	3.92	0.047	< 1
Degree of deciduousness (DOD)	82.19	4.41	0.036	< 1
Proximity to historical disturbance (HD)	82.04	4.26	0.039	< 1
Proximity to contemporary disturbance (CD)	82.26	4.48	0.035	< 1
Proximity to edge (EDGE)	82.42	4.64	0.032	< 1
<i>L. camara</i> neighbourhood index (LNI)	77.78	0.00	0.330	6.19
FF:DOD	84.39	6.61	0.012	1.60
FF:HD	84.56	6.78	0.011	1.34
FF:CD	84.61	6.83	0.011	1.27
FF:EDGE	84.82	7.04	0.010	< 1
DOD:HD	84.30	6.52	0.013	1.69
DOD:CD	85.13	7.36	0.008	< 1
DOD:EDGE	85.27	7.49	0.008	< 1
HD:CD	85.04	7.26	0.009	< 1
HD:EDGE	85.16	7.39	0.008	< 1
CD:EDGE	85.36	7.59	0.007	< 1
LNI:FF	80.44	2.66	0.087	6.81
LNI:DOD	80.68	2.90	0.077	6.50
LNI:HD	79.96	2.18	0.111	7.45
LNI:CD	80.02	2.24	0.108	7.38
LNI:EDGE	123.41	45.63	0.000	7.24

colonisation when compared to the *L. camara* neighbourhood index alone. The global model (all predictors individually, and all two-way additive interactions of predictors) explained 51.1% of the deviance from the intercept-only model.

Lantana camara change in density

The spatial extent of *L. camara* in BRT increased dramatically from 1997 to 2008. *Lantana camara* was present in 41% of plots across the 540 km² of BRT in 1997 (Murali and Setty 2001), but had spread to 81% of plots by 2008; this doubling in spatial extent was accompanied by a more than 3-fold increase in density (Sundaram and Hiremath 2012). Of the total 51 plots that were invaded in 1997, *L. camara* density increased in 37 plots, remained the same in 2 plots, and decreased in 12 plots.

The global model containing all predictors and their interactions explained 56.8% of the deviance from the intercept-only model for *L. camara* density data. The

Table 2. Models to explain *Lantana camara* change in density with their corresponding AIC_c (corrected AIC), Δ_i (value of AIC_c in the i^{th} model – minimum value of AIC_c), Akaike weights and percent deviance explained. The single-predictor model explaining the maximum deviance is in bold typeface.

Model	AIC _c	Δ_i	Akaike weights	% deviance explained
<i>L. camara</i> density (n=51)				
Global	219.71	50.76	0.000	56.75
Fire frequency (FF)	168.94	0.00	0.400	23.59
Degree of deciduousness (DOD)	182.67	13.72	0.000	< 1
Proximity to historical disturbance (HD)	182.51	13.57	0.000	< 1
Proximity to contemporary disturbance (CD)	182.64	13.70	0.000	< 1
Proximity to edge (EDGE)	181.98	13.03	0.001	1.35
<i>L. camara</i> neighbourhood index (LNI)	182.36	13.42	0.000	< 1
FF:DOD	172.12	3.17	0.083	23.69
FF:HD	171.27	2.32	0.127	24.95
FF:CD	172.15	3.20	0.082	23.65
FF:EDGE	170.31	1.37	0.204	26.35
DOD:HD	185.69	16.74	0.000	< 1
DOD:CD	185.87	16.93	0.000	< 1
DOD:EDGE	185.18	16.24	0.000	1.41
HD:CD	185.71	16.77	0.000	< 1
HD:EDGE	185.11	16.16	0.000	1.56
CD:EDGE	185.21	16.27	0.000	1.36
LNI:FF	171.74	2.79	0.100	24.26
LNI:DOD	185.60	16.65	0.000	< 1
LNI:HD	185.27	16.32	0.000	1.25
LNI:CD	185.57	16.63	0.000	< 1
LNI:EDGE	184.94	16.00	0.000	1.88

Table 3. Weight of evidence in favour of each variable for explaining *L. camara* colonisation and change in density. The weights for each variable were calculated by summing the Akaike weights of all models where the predictor variable of interest appears (Burnham and Anderson 2002). Greater weights (highlighted) are indicative of the most important predictor, relative to all others.

Predictor variables	Colonisation	Change in density
Fire frequency	0.12	0.62
Degree of deciduousness	0.10	0.05
Proximity to historical disturbance	0.13	0.08
Proximity to contemporary disturbance	0.12	0.05
Proximity to edge	0.04	0.13
<i>L. camara</i> neighbourhood index	0.48	0.06

frequency of fire in each grid cell during 1997–2008 emerged as the best predictor of change in *L. camara* density and explained 23.6% of the deviance from the intercept-only model for the data (Table 2). Between 1997 and 2008, 22 plots were

unburned, 19 plots had one instance of fire, 6 plots had two instances of fire, and 4 plots had three instances of fire. Contrary to expectation, change in *L. camara* density and fire frequency appeared to be negatively related. An increase in fire frequency was accompanied by a decrease, rather than an increase in *L. camara* density. Fire frequency and change in *L. camara* density appeared to be negatively related, although we have very few plots that burned more than two times to be able to assert this trend as definitive (Fig. 2b). Summing the Akaike weights across all models where fire frequency appeared as a predictor revealed that fire frequency had the highest evidence of support when compared to all other predictors (Table 3). Although there was a positive relationship between edge and change in *L. camara* density, edge was a distant second to fire frequency in terms of the percent deviance explained (Table 2). The weight of evidence in favour of edge was also low compared with that of fire frequency (Table 3).

Discussion

Results from this study indicate that the factors that are important for *L. camara* invasion differ across stages. Propagule pressure (as inferred from the *L. camara* neighbourhood index) plays an important role in increasing the probability of local colonization of *L. camara*. Following *L. camara* colonisation, fire appears to limit *L. camara* density.

While two factors have emerged as being important mechanisms of the *L. camara* invasion process, the three other disturbance factors taken into account in this study (distance to historical disturbance, distance to current human habitation, and distance to edge) and *L. camara* habitat suitability, also play a role. The absence of interactions among our predictor variables was surprising, given our initial expectation of synergistic interaction effects between predictors.

In addition to the factors identified by this study as potential underlying mechanisms of *L. camara* colonisation and change in density, contemporary reports of *L. camara* proliferation in the larger Western Ghats landscape, e.g., in nearby protected areas like Bandipur (Prasad 2010) and Mudumalai (Ramaswami et al. 2014) indicate the possible role of other mechanisms operating at a larger scale, e.g., extended drought or stochastic rainfall. Additionally, the effect of other local-scale factors e.g., biotic interactions, soil, and topography, as possible mechanisms underlying invasion have not been investigated by this study.

Lantana camara colonisation and the importance of the *L. camara* neighbourhood index

In BRT, *L. camara* produces large fruit crops, sometimes up to ten thousand fruits per plant over a single fruiting season (Monika Kaushik, unpublished data). It is likely that arrival of *L. camara* propagules is enhanced by the year-round fruiting of

the species combined with the lack of dispersal limitation. Indeed, studies on the Island of Reunion have shown that larger populations of dispersers (e.g., the invasive red-whiskered bulbul) are supported in areas invaded by four bird-dispersed invasive plants that produce seeds year-round (including *L. camara*) compared to areas with a low invasive plant density. This suggests a positive feedback between presence of propagules and presence of dispersers (Mandon-Dalger et al. 2004). Bulbuls are responsible for the dispersal of *L. camara* seeds in India as well, with *L. camara* fruits forming up to 10% of their diet (Bhatt and Kumar 2001). Studies from Hong Kong show that *L. camara*'s sugar-rich berries also attract other birds (e.g., light-vented bulbul; Corlett 1998). Preliminary studies from our site show that frugivorous bird density and diversity is higher in areas with high lantana fruit density, indicating that birds could be important dispersers of *L. camara* fruits in these forests as well (Monika Kaushik, unpublished).

In addition to its dispersal by frugivorous birds, we have observed *L. camara* seeds in feces of wild pigs and sloth bears in BRT, although there are no published records of *L. camara* seed dispersal by mammals. In addition to sexual modes of propagation, it should also be noted that *L. camara* propagates vegetatively through rootstock (Day et al. 2003), implying that the non-detection of stems aboveground does not necessarily imply the absence of *L. camara* in a given site as rootstock may regenerate when favourable conditions (e.g., a rainfall event) occurs.

While the *L. camara* neighbourhood index emerged as the primary driver of *L. camara* colonisation, other predictors were not negligible, nor sizeable, and the global model explained 51.1% of the variance in the data. The effects of predictors associated with disturbance, such as distance to edge, fire frequency, and distance to both contemporary and historical disturbance, were almost similar. Both Daehler (1998) and Colautti et al. (2006) observe that synergy between factors that influence both the invasiveness of species and the invasibility of landscapes is the rule, rather than the exception. Although this points to a synergy between factors that promote invasiveness (propagule pressure, or rather the *L. camara* neighbourhood index, in this case) and ecosystem invasibility (fire frequency, disturbance and edge), we were unable to detect interactions between predictor pairs.

On the other hand, the relatively lower weight of evidence in favour of degree of deciduousness when compared to the *L. camara* neighbourhood index (and also edge and fire frequency) may be related to the relative representation of different forest types within the BRT landscape. Although a large proportion of our study area is deciduous (~90%; Krishnaswamy et al. 2009) the degree of deciduousness is a continuous variable that spans the continuum in phenological and tree density variation across different types of tropical deciduous forest and savanna-woodland vegetation types (Krishnaswamy et al. 2009, Krishnaswamy 2013). We thus expected that degree of deciduousness would be a good predictor of *L. camara* colonisation, since *L. camara* is known to be sensitive to moisture availability and canopy openness (Sharma et al. 2005). However, it could be that the scale at which the degree of deciduousness was calculated differs from the scale at which *L. camara* colonises habitat.

Fire as a factor limiting *L. camara* change in density

Results from our study have improved our understanding of the relationship between *L. camara* and fire. Some conceptual models of *L. camara* invasibility have hypothesized that an increase in fire frequency could potentially favour *L. camara* (Hiremath and Sundaram 2005, Sharma et al. 2005). More recent work (Sundaram et al. 2012, this study) suggests the opposite, that *L. camara* density may be depressed by fire. Our results are consistent with observed fire effects on *L. camara* in Australia (Day et al. 2003, Debus and Lewis 2014), i.e., that frequent fires prevent, rather than encourage, *L. camara* spread. We also have evidence to suggest that fires may reduce the density of viable *L. camara* seeds in the soil seed bank (Hiremath and Sundaram 2013).

Just as the role of fire in limiting *L. camara* density was contrary to our expectations, so too was the minimal role of degree of deciduousness and the *L. camara* neighbourhood index in influencing *L. camara* density. Studies from Australia and Africa show that *L. camara* change in density is enhanced by the presence of open canopies or gaps (Totland et al. 2005). In our case, the effects of degree of deciduousness are probably masked by the large spatial extent of deciduous forests in BRT compared to other forest types.

Propagule pressure – as inferred from the *L. camara* neighbourhood index in this case – presumably does not play much of a role in influencing *L. camara* density because allochthonous dispersal (i.e., arrival of seeds from outside) may cease to be important following the colonisation of *L. camara* at a site. Given the young age to maturity of *L. camara* and the large numbers of fruits produced per individual, resulting autochthonous seed arrival (i.e., seeds produced on-site) may swamp the effects of seeds arriving from elsewhere.

Suppression of forest fires and windows of opportunity for *L. camara* invasion

Davis et al. (2000) point out the complex nature of the invasion process resulting from its dependence on intermittent disturbance. Additionally, these disturbances must coincide with propagule availability in order to increase the possibility of successful invasion. In the case of *L. camara*, change in the disturbance regime caused by the suppression of forest fires has probably increased invasibility of some ecosystems (Sundaram et al. 2012) with propagule pressure exerted by the soil seed bank (Hiremath and Sundaram 2013, Debus and Lewis 2014) as the causal mechanistic link. Ecosystem susceptibility to invasion is therefore not a static or permanent attribute, but one that fluctuates over time depending on favourable conditions being created (Davis et al. 2000), and contingent on propagule pressure being present to make use of windows of opportunity.

In Australia and in India, *L. camara* invasion is probably driven by the capacity of propagule pressure to overwhelm ecological resistance to invasion. D'Antonio et al. (2001) decompose ecological resistance into abiotic (climate conditions, soil

characteristics) and biotic (competition, parasitism, pathogen attack) components and further suggest that when ecological resistance to invasion is high (for example, in areas where fires are a regular occurrence), invasion would occur only when propagule pressure is high, or when invaders themselves can alter resistance. Similar to Davis et al. (2000), D'Antonio et al. (2001) also theorize that high ecological resistance to invasion could be overwhelmed by low, but steady, supply of propagules in space and time, allowing for the exploitation of windows of opportunity.

Management implications

Lantana camara invasion in BRT is the product of a complex interplay between propagule pressure and frequency of forest fires. Clearly, therefore, land managers and biologists need to take into account the inherent multivariate nature of *L. camara* invasion when coordinating eradication or control activities.

Once a species is established, the reduction of propagule pressure is a challenging task (Lockwood et al. 2005). Limiting propagule pressure by physical removal of *L. camara* has been found to be successful, e.g., in Australia (Day et al. 2003) and South Africa (van Wilgen et al. 2004), although costs of removal are prohibitive when the area of operation is large. Additionally, *L. camara* removal has to be conducted repeatedly because of *L. camara* re-growth (Day et al. 2003, Babu et al. 2009, Ramaswami et al. 2014). Prioritizing *L. camara* removal activities in heavily invaded areas could help reduce propagule pressure in the long run (Chornesky et al. 2005), provided *L. camara* colonisation and density are contained in areas that are currently less invaded.

In addition to propagule pressure, our finding that fire limits *L. camara* density also has important management implications. In the Indian context, fires have been regarded by forest managers as uniformly detrimental (Bahuguna and Upadhyay 2002). However, *L. camara* control efforts elsewhere have successfully used fire as a cheap and effective management tool (Day et al. 2003, Debuse and Lewis 2014). On a cautionary note, the use of fire could cause undesirable damage to native trees and other biodiversity components due to abundant fuel provided by *L. camara*. However, using regulated fire as a management tool could be considered if *L. camara* abundance and density could be first reduced by physical removal. Forest managers have attempted physical removals of *L. camara*, but without repeated removals over large areas, removal efforts have largely failed (Sundaram et al. 2012). *Lantana camara* control through fire may be more feasible in deciduous forests, assuming fuel loads could be lowered and kept low.

Acknowledgements

We thank the staff of the Ashoka Trust for Research in Ecology and the Environment (ATREE) BRT Field Station, for their logistical support and help with field data collection, Devcharan Jathanna and Kavita Isvaran for their help with data analysis, and

Mahesh Sankaran, Varun Verma, Noelie Maurel, and two anonymous reviewers for their constructive comments. We thank R. Siddappa Setty for sharing data on fire frequency and *L. camara* distribution, C. Made Gowda and Nitin Rai for sharing data on historical habitation sites, MC Kiran for collating fire frequency data, and Madhura Niphadkar for creating maps used in this study. The Department of Science and Technology, India, and the International Foundation for Science, Sweden, funded this study.

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The thin green line: sustainable bioenergy feedstocks or invaders in waiting

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Academic editor: J. Molofsky | Received 17 September 2014 | Accepted 10 January 2015 | Published 14 April 2015

Citation: Smith LL, Allen DJ, Barney JN (2015) The thin green line: sustainable bioenergy feedstocks or invaders in waiting. NeoBiota 25: 47–71. doi: 10.3897/neobiota.25.8613

Abstract

Numerous fast growing and highly competitive exotic crops are being selected for production of renewable bioenergy. Tolerance of poor growing conditions with minimal inputs are ideal characteristics for bioenergy feedstocks, but have attracted concern for their potential to become invasive. *Miscanthus × giganteus* is one of the most promising bioenergy crops in the US, but grower adoption is hindered by high establishment costs due to sterility. Newly developed fertile tetraploid *M. × giganteus* may streamline cultivation while reducing establishment costs. However, fertile seed dramatically increases the potential propagule pressure, and thus probability of off-site plant establishment. To empirically evaluate the invasive potential of fertile *M. × giganteus* in the Southeastern US, we compared fitness and spread potential relative to ten grass species comprising 19 accessions under both high and low levels of competition and disturbance. We chose species known to be invasive in the US (positive controls: *Arundo donax*, naturalized *M. sinensis*, *M. sacchariflorus*, *Phalaris arundinacea*, *Sorghum halepense*) and non-invasive (negative controls; *Andropogon gerardii*, ornamental *M. sinensis*, *Panicum virgatum*, *Sorghum bicolor*, *Saccharum* spp.). This novel design allows us to make relative comparisons of risk among species with varying invasiveness. After three years of establishment and growth in Blacksburg, Virginia, neither aboveground disturbance nor interspecific weed competition influenced fitness for fertile *M. × giganteus* or our positive and negative control groups. Fertile *M. × giganteus* produced 346% and 283% greater aboveground biomass than our positive and negative species, respectively. However, fertile *M. × giganteus* produced 74% fewer inflorescences m⁻² than our positive controls and 7% and 51% fewer spikelets inflorescence⁻¹ than the positive and negative control species. After 18 months of growth, we observed the vegetative and seedling spread of three of our positive control species (*S. halepense*, *P. arundinacea*, and *M. sacchariflorus*) outside the cultivated plot

into receiving areas of both high and low competition. After 24 months of growth, numerous species were observed outside the cultivated plot including fertile *M. × giganteus* and 50% of negative control species. Notably, in three years sterile *M. × giganteus* 'Illinois' and *Arundo donax* never moved from the cultivated plot. The addition of fertile seed appears to increase the potential for offsite movement, but within the geographic confines of our empirical evaluation, fertile *M. × giganteus* seedlings are more similar to native *P. virgatum* and were not nearly as fast growing or as competitive as our positive control *S. halepense*. The use of numerous species providing relative comparisons allow us to draw important conclusions which may help prepare for widespread commercialization, while providing novel methodology for ecological risk assessment of new species.

Keywords

Biofuel, giant miscanthus, habitat susceptibility, invasibility

Introduction

There is a global push towards renewable biomass based energy (Yauan et al. 2008), and large statured perennial grasses hold the most promise as dedicated energy crops. Candidate feedstocks are ideal because of their perennial growth habit, rapid growth and high annual biomass production, low management and input requirements following establishment, and relatively low pest pressure. However, it is this desirable set of agronomic characteristics which has been the major source of concern for their potential to contribute to the invasiveness of numerous bioenergy crops (Lewandowski et al. 2003; Raghu et al. 2006). Barney and DiTomaso (2010) diagram the “thin green line” between many agronomic weeds, introduced and even subsidized in some cases, for purposes such as forage or erosion control, and the relatively benign crops vitally important to our economy and food supply. However, identifying which side of this line new crops fall is challenging at best.

Spatial demographic models (Matlaga and Davis 2013) and weed risk assessments (Barney and DiTomaso 2008; Davis et al. 2010; Gordon et al. 2011) do offer predictions regarding the ability of novel species to establish and spread. However, in reality, empirical data from in situ field trials, supporting conclusions of invasiveness or long-term sustainability, do not exist. Several candidate bioenergy crops have a history of invasiveness, which is a robust predictor of future invasive potential (Dawson et al. 2009; Gordon et al. 2008). For example, *Arundo donax* L. is a documented noxious weed of riparian habitats in the southeastern United States (Bell 1997; Katibah 1984). Despite this label, no peer-reviewed data exists evaluating the ability of *A. donax* to spread from a cultivated field. Two miscanthus species in particular have been widely grown in the United States for horticultural use: *Miscanthus sinensis* Andersson is listed as potentially invasive, but not prohibited in Connecticut (Council 2013), and is known to form extensive infestations after spreading from older or abandoned ornamental plantings (Dougherty et al. 2014; Miller 2003); and *M. sacchariflorus* (Maxim.) Franch is on the Massachusetts prohibited plant list (Resources 2014), and has repeatedly escaped from cultivation, particularly in the Midwest (Bonin et al. 2014). However, at this time only

limited studies have begun to examine the invasive potential of bioenergy crops in the context of a managed agricultural cropping system (see Barney et al. 2012; Matlaga et al. 2012b; Quinn et al. 2011).

Crop breeding and improvement will be imperative to improve quality, increase yield, and reduce pest pressure (Gressel 2008). The sterile triploid 'Illinois' variety of *Miscanthus* \times *giganteus* J.M. Greef & Deuter ex Hodkinson & Renvoize has emerged as one of the most promising bioenergy crops in the US and Europe, but planting is expensive and requires specialized equipment (Lewandowski et al. 2003). Despite the explicit inclusion of *M. x giganteus* in the Massachusetts prohibited plant list as a progeny of *M. sacchariflorus* (Resources 2014), qualitative weed risk assessments have suggested that the sterile cultivar is of low risk for invasiveness (Barney and DiTomaso 2008). Newly developed fertile lines of tetraploid *M. x giganteus* may streamline cultivation by reducing labor and establishment costs (Sacks et al. 2013); but the addition of fertile seed has the potential to dramatically increase propagule pressure to surrounding habitats, and must be evaluated for its influence on invasiveness.

Despite the vigilant approach with bioenergy crops in regards to invasiveness, the majority of introduced species have neutral ecological consequences and many provide a direct benefit to society (Barney and DiTomaso 2010; Davis 2003). However, the intentional cultivation and transport of exotic bioenergy crops over a vast geographic range would bypass the early environmental filters of introduction and colonization (Barney et al. 2012), as well as the geographical, environmental and reproductive barriers to spread (Richardson and Blanchard 2011; Smith and Barney 2014b). Both vegetative and seed propagules from bioenergy crops will be exposed to a diversity of landscapes along the biofuel supply chain (cultivated field to refinery). Therefore, susceptibility to invasion will need to be evaluated across numerous geographies and habitat types (Smith and Barney 2014b).

Since Herbert Baker (1965; 1974) put forth the theory that a set of 12 defining characteristics could identify the 'ideal weed', the importance of traits has been widely debated (Thompson and Davis 2011; van Kleunen et al. 2010). Bioenergy crops have been selected for a suite of agronomic traits making them ideal candidates for cultivation (Lewandowski et al. 2003), but this may also serve as the crux for their potential to become invasive (Raghu et al. 2006). For these reasons, it is imperative that we reflect that no single species is invasive in every location it inhabits. For example, populations of *Sorghum halepense* L. are particularly devastating in the southeastern United States, earning a reputation as one of the world's worst weeds (Holm et al. 1977). Yet in its northern range, *S. halepense* populations are rarely regarded as detrimental and despite its perennial growth many populations do not overwinter as rhizomes (Warwick et al. 1984), illustrating that both invasiveness and habitat susceptibility vary (Smith and Barney 2014b).

Here we use a comparative framework to relativize the invasive potential of newly developed fertile tetraploid *M. x giganteus*. We compare fertile *M. x giganteus* against ten grass species, comprising 19 accessions, in four environments. We selected the ten grass species to allow a comparison against species that are known invaders in the

US (positive controls), and species that are generally considered not to be invasive (negative controls). This design allows us to make important relative comparisons of risk, for candidate bioenergy crops, along a spectrum of invasiveness. We impose both competition and aboveground disturbance treatments to capture a range of conditions which bioenergy crops may encounter in or adjacent to the cultivated field. These treatments allow us to determine conditions that facilitate invasive spread and determine susceptible environments for establishment of nascent populations. This relative methodology was recently tested and proved critical in accurately interpreting the probability of fertile tetraploid *M. × giganteus* establishment in a diversity of habitats across the southeastern United States (Smith and Barney 2014b). The objective of this study is to compare the growth and spread potential of fertile *M. × giganteus* to known invasive and noninvasive control groupings. Specifically, we aim to: (1) evaluate the invasive potential of fertile *M. × giganteus*, during the first three years of establishment and growth, in comparison with 10 species (19 total accessions) of known invasive and non-invasive species in relation to their population dynamics, competitive ability, local recruitment and spread potential; (2) evaluate the performance of each bioenergy crop in response to various levels of competition and disturbance by assessing survival and performance; and (3) quantify seed production as a novel propagule source for *M. × giganteus* and compare across our invasiveness diversity panel.

Methods

Species selection

In our effort to evaluate the invasive potential of a new fertile tetraploid *M. × giganteus* pre-commercial cultivar known as ‘PowerCane’ ((Sacks et al. 2013); Mendel Biotechnology, Inc., Hayward, CA, USA), which was derived from a population of half-siblings of ‘Nagara’ (Głowacka et al. 2014), we developed a methodology that includes several species that are known invaders in the southeastern US (hereafter positive controls), as well as species that are generally considered not invasive (hereafter negative controls). In order to make relative comparisons of fertile *M. × giganteus*, we selected a range of grasses to represent various growth habits (clumping to spreading) and fertilities that span this invasive spectrum, and that occur regionally (Table 1). Weedy populations of all positive controls are well documented regional invaders (Table 1), and many receive a high risk rating from the Plant Protection and Quarantine Weed Risk Assessment Model (Koop et al. 2011; Smith 2014). Negative controls have been documented as low risk in weed risk assessment models (PIER 2013; Smith 2014), or are native to North America and are not considered highly competitive or weedy in comparison to our positive controls (Simberloff et al. 2012). In some cases multiple accessions (e.g., *M. sinensis*) or cultivars (e.g., *P. virgatum*) are included to represent intraspecific variation (Table 1). Despite the weedy reputation of *M. sinensis* (Dougherty et al. 2014; Miller 2003) we selected accessions intended to represent both positive and negative

Table 1. List of taxa included in the field trials located in Blacksburg, Virginia.

Species	Common name	Accession	Source	Planting method	Planting format [§]	Invasive status in the US
<i>Andropogon gerardii</i>	big bluestem	Suther	Ernst	seed	16.5 R	native [†]
<i>Arundo donax</i>	giant reed		Bluemel	plugs	76 C	invasive [‡]
<i>Miscanthus sacchariflorus</i>	Amur silvergrass	Robustus	Bluemel	plugs	76 C	invasive [#]
<i>M. sinensis</i>	maiden grass	Gracillimus	Bluemel	plugs	76 C	exotic ^{††}
<i>M. sinensis</i>	maiden grass	Dixieland	Bluemel	plugs	76 C	exotic ^{††}
<i>M. sinensis</i>	maiden grass	Cabaret	Bluemel	plugs	76 C	exotic ^{††}
<i>M. sinensis</i>	maiden grass	OH	UIUC	plugs	76 C	invasive ^{‡‡}
<i>M. sinensis</i>	maiden grass	NC	UIUC	plugs	76 C	invasive ^{‡‡}
<i>M. sinensis</i>	maiden grass	KY	UIUC	plugs	76 C	invasive ^{‡‡}
<i>M. sinensis</i>	maiden grass	PA	UIUC	plugs	76 C	invasive ^{‡‡}
<i>M. × giganteus</i>	giant miscanthus	Illinois	Mendel	plugs	76 C	exotic ^{§§}
<i>M. × giganteus</i> [†]	PowerCane	M700464	Mendel	plugs	76 C	unknown
<i>M. × giganteus</i>	giant miscanthus	Nagara	Mendel	plugs	76 C	exotic ^{§§}
<i>Panicum virgatum</i>	switchgrass	EG 1101	Ceres	seed	16.5 R	native [†]
<i>P. virgatum</i>	switchgrass	Alamo	Ernst	seed	16.5 R	native [†]
<i>Phalaris arundinacea</i>	reed canarygrass	Palaton	Outsidepride	seed	16.5 R	invasive
<i>Saccharum</i> spp. [‡]	energy cane	US 06-9001	USDA-ARS	ratoons	76 C	exotic ^{‡‡}
<i>Saccharum</i> spp.	energy cane	US 06-9002	USDA-ARS	ratoons	76 C	exotic ^{‡‡}
<i>Sorghum bicolor</i>	biomass sorghum	ES 5201	Ceres	seed	76 R	exotic ^{##}
<i>S. halepense</i>	johnsongrass		Azlin	seed	16.5 R	invasive ^{†††}

[†] Following the nothospecies rule from the International Code of Nomenclature for algae, fungi, and plants (IAPT 2012), all progeny and other descendants derived from crossing *M. sacchariflorus* and *M. sinensis* are by definition *M. × giganteus*, irrespective of ploidy and fertility.

[‡] Due to issues with availability, *Saccharum* cultivars were planted in summer 2012, one year later than all other species.

[§] Planting format in 16.5 cm (16.5 R) or 76 cm (76 R) rows, or on 76 cm centers (76 C).

^{||}(Simberloff et al. 2012); [‡](Bell 1997); [#](Bonin et al. 2014); ^{††}(Madeja et al. 2012); ^{‡‡}(Dougherty et al. 2014); ^{§§}(Heaton et al. 2004); ^{||}(Kercher et al. 2007); ^{‡‡}(Gordon et al. 2011); ^{##}(Martin et al. 2006);

^{†††}(Holm et al. 1977).

controls. Several *M. sinensis* ornamental cultivars are poor horticultural performers with low fecundity (Madeja et al. 2012), hence our characterization of low invasive potential in this study.

Experimental design

A two-factor split-plot design arranged in a randomized complete block, with four replications was established in Blacksburg, Virginia, Schochoh, Kentucky and Auburn, Alabama in 2011. A total of 20 13.7 × 18.3 m plots were established for each accession with the exception of the four naturalized *M. sinensis* accessions, which were replicated three times at each site due to seed limitation. Within each plot we planted the center

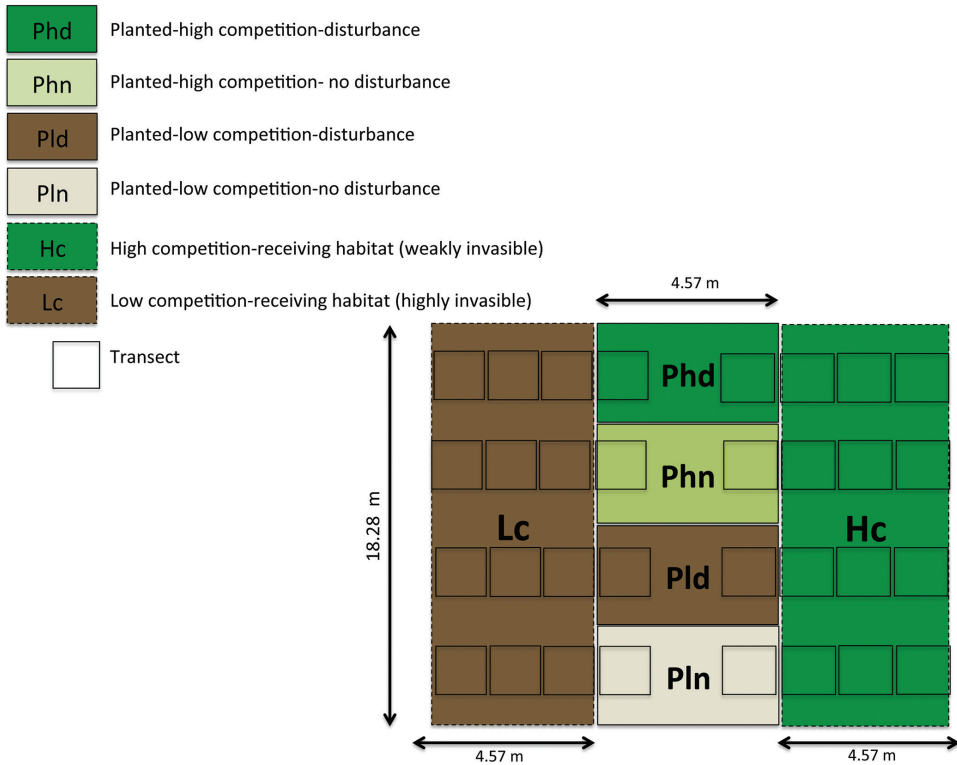


Figure 1. A single replicate, showing the two-factor split-plot layout for each of the 76 plots established in Blacksburg, VA.

4.6×18.3 m with the target taxon, which is flanked by an equally-sized receiving area on either side. The planted plot was divided into four 4.6×4.6 m subplots (20.88 m^2) randomly assigned to one of the following treatments (Figure 1): high competition/ no disturbance (Phn); high competition/ disturbance (Phd); low competition/ no disturbance (Pln); low competition/ disturbance (Pld). The disturbance treatment refers to annual aboveground biomass removal in the fall of each year (beginning December 2011), while a no disturbance treatment is defined by the absence of annual harvest, in which all plant material was left standing in the field for the duration of this study. The high competition treatment refers to no weed management action taken following initial establishment (after July 2011). High competition plots included dense populations of both winter (i.e., *Lamium* sp. and *Stellaria* sp.) and summer annual weeds (i.e., *Ipomoea* sp., *Amaranthus* sp., *Setaria* sp., and *Conyza canadensis* (L.) Cronquist). Low competition is defined as intensive weed management to maintain a near weed-free (-bare ground) environment throughout the duration of the experiment. Each flanking unplanted 4.6×18.3 m “receiving habitat” was also randomly assigned to either intensive management for weeds (Lc) or no management (Hc) (Figure 1), to assess the local spread of each species as a function of habitat type. The Hc receiving areas

were composed of the same winter and summer annual weeds as above. Weedy plots represent a relatively high competition habitat, while the weed-free plots represent a relatively low competition habitat. Each plot was surrounded with a 1.5 m buffer of mowed *Schedonorus arundinaceus* (Schreb.) Dumort (tall fescue).

Site

This experiment was established at the Kentland Research Farm near Blacksburg, VA USA (37°12'N, 80°35'W), on 10 June 2011, Walnut Grove Farms, Schochoh, KY (36°45'N, 86°45'W) on 15 June 2011, and Auburn, Alabama on 30 May 2011 (32°26'N, 85°52'W). However, due to unforeseen circumstances beyond our control, the Kentucky and Alabama locations were eradicated within the first year of the study. Therefore, neither will be discussed further. We understand the limitations of a single geographic location in years two and three of this study; but due to the proprietary nature of the 'PowerCane' and 'Nagara' plant material, we were limited by site availability. It should be noted that other important ecological studies have been carried out using a single location (Von Holle 2005; Von Holle and Simberloff 2005). However, our results should be viewed within the limited geographic representation. Soil samples were collected in early June 2011, using a 20 cm³ soil corer, and submitted for analysis at the Virginia Cooperative Extension Soil Testing Laboratory at Virginia Tech. The Blacksburg field site was planted on a Ross loam occluding a Wheeling silt loam (USDA-NRCS 2013) with a pH of 6.4, and a recent cropping history of continuous corn with a winter rye cover crop. In May 2011 the field was treated with 1 kg acid equivalent ha⁻¹ glyphosate. The rye cover crop was mowed and bailed in preparation for planting.

Establishment

Vegetatively propagated accessions (Table 1) were started in the greenhouse from rhizome/root crown fragments and delivered to the field sites just prior to planting. Seed from naturalized accessions of *M. sinensis* were collected the previous year from established populations in Kentucky, Pennsylvania, North Carolina and Ohio. Seeds were planted individually into 127 cell flats in March 2011 and greenhouse grown. The field was not tilled prior to planting in accordance with a no-till cropping system. Miscanthus plugs were planted on 76 cm centers using a no-till plug planter (RJ Equipment, Ontario, Canada). Larger plant material such as *A. donax* required hand planting, because pieces were too large for the transplanting equipment. All remaining seeded feedstocks were drilled on 16.5 or 76 cm rows using a no-till drill (Table 1). Seeding rate was adjusted based on commercially determined pure live seed or (laboratory) germination rates, to deliver ~22,000 plants ha⁻¹, which was comparable to our transplant density. As our study aims to evaluate establishment, persistence and spread,

annual *Sorghum bicolor* L. was not replanted annually after June 2011. *Saccharum* US 06-9001 and US 06-9002 were not planted until May 2012, due to delays in germ-plasm availability.

To improve stand establishment, the entire field site received 350 g ai ha⁻¹ and 822 g ai ha⁻¹ 2,4-D on July 6 and July 25 respectively. Following the July herbicide application we decided that sufficient time for seedling/plug establishment had elapsed, and thus no further herbicide applications were made in the high competition plots (Phn and Phd). No herbicide treatments were imposed in the high competition receiving habitat (Hc). Herbicide treatments of 1060 g ai ha⁻¹ 2,4-D plus 560 g ai ha⁻¹ dicamba were sprayed on August 20, 2011, in the low competition plots (Pld and Pln) and low competition receiving habitats (Lc). Supplemental hand weeding was done as needed. A second treatment of 2,4-D and dicamba (1060 g ai ha⁻¹ and 560 g ai ha⁻¹ respectively) was applied on September 25. Low competition plots received 1680 g ai ha⁻¹ atrazine at the beginning of the second and third growing seasons. Herbicide treatments of 1060 g ai ha⁻¹ 2,4-D plus 560 g ai ha⁻¹ dicamba and 31.5 g ai ha⁻¹ halosulfuron including a 1% v/v nonionic surfactant were applied approximately once a month to maintain weed free status within plots and in the low competition receiving habitat. A 1 kg ae ha⁻¹ application of glyphosate was also used to selectively spot treat non-target grass weeds when hand weeding was not time effective.

Measurements

Spring data collection occurred in May of 2012 and was repeated in May 2013, while fall data collection occurred in November of each year prior to harvest. To characterize population demography, seedling recruitment and individual plant performance, we placed two 0.9 × 1.2 m quadrats in the middle of each sub-plot adjacent to the receiving habitat (Figure 1). Previous germination studies of *M. × giganteus* ‘Illinois’ failed to yield evidence of fertile seed (Matlaga et al. 2012b); thus, any *Miscanthus* seedlings observed within our plots of *M. × giganteus* ‘Illinois’ and ‘Nagara’, both of which are sterile, were broadly designated *Miscanthus* spp. and assumed to have moved beyond the 4.6 × 18.3 m receiving area of *Miscanthus* spp. Due to the large number of visually identical *Miscanthus* spp. seedlings and lack of available tools for determining genetic lineage, we made the assumption that any *Miscanthus* seedling found within a seed bearing *Miscanthus* (*M. sinensis* or ‘PowerCane’) plot or receiving area was the progeny of plants associated with that specific plot. The same assumption was also made for the two cultivars of *P. virgatum*. Measurements include plant density, canopy height to the tallest node, culm number per plant, number of inflorescences per plant and basal plant diameter. After two growing seasons the habit of *S. halepense*, *M. sacchariflorus* and *P. arundinacea* made it impossible to distinguish vegetative ramets from adjacent plants or seedlings, hence our need to base all measurements on culms per unit area rather than the number of individuals. In the receiving habitats, data was collected in

three 0.9×1.2 -m quadrats arranged as a transect perpendicular to the planted plot, adjacent to the planted area until the end of the 4.57 m receiving habitat (Figure 1). The same metrics as above were collected for newly emerged plants found in the receiving habitat (of any of the 20 accessions in this study). Five inflorescences were randomly harvested from each subplot in November 2013. Total spikelet number for each inflorescence was recorded with the exception of the sterile *A. donax*, *M. × giganteus* ‘Illinois’, and *M. × giganteus* ‘Nagara’ (Mariani et al. 2010; Matlaga et al. 2012a). Three replications of 100 seeds for each “fertile” accession were surface sterilized with 10% bleach for 30 seconds and rinsed with deionized water. Seeds were then placed on germination paper in 9.5×9.5 cm sealed petri plates with ~10 ml deionized water and germination was monitored for four weeks. Harvest treatments were imposed from November to January in each year as weather permitted.

Follow up

Upon termination of the experiment, the entire field was sprayed with 2 kg ae ha⁻¹ glyphosate in late 2013 and early 2014. All plant material was harvested, removed from the site and burned as was done with the harvested material in 2012 and 2013. In late summer 2014 we applied 7 L ha⁻¹ imazapyr. Glyphosate-tolerant corn or soybeans will be planted in the spring of 2015, and a three-year scouting and weed management plan will be implemented to ensure all propagules have been removed from the site. It should be noted that no individuals of any species have been detected outside the experimental area to date.

Statistical analysis

Analysis of variance (ANOVA) was performed on fitness parameters using JMP 10 statistical software (SAS Institute, Cary, North Carolina, USA). Aboveground biomass, height, culm number, inflorescence number, seed number, and seedling density were analyzed as a mixed model. Treatments and accessions are considered fixed effects, with the 20 accessions nested within designated invasiveness groups (positive and negative controls), while blocks were considered a random effect. Numerous transformations were performed, depending on measurement and year, to achieve normality of residuals. All interactions varied by year, and we were only interested in within year comparisons. Therefore, we did not perform a repeated measures analysis, and look at the variance structure within each of the three years. When significant treatment effects occurred, means were compared with Tukey-Kramer test at $\alpha < 0.05$, or when more complex interactions were significant, means were compared with a priori orthogonal contrasts at $\alpha < 0.05$. The 20 individual accessions in our study had an underlying structure (invasiveness groupings), central to our

experimental design. To objectively determine if our measured traits were capable of partitioning the accessions into the invasiveness groups we performed a canonical discriminant analysis. Kenkel et al. (2002) suggest canonical discriminant analysis is appropriate to examine the relationships between our transformed fitness metrics height, culm and inflorescence number, and spikelet production (covariates) and our invasiveness response variables.

Results

Establishment (year 1)

Growth in the first year of all 19 perennial grasses was low as expected. Establishment was well below our target density of 22,000 plants ha⁻¹ for the negative controls *A. gerardii* and ornamental cultivars of *M. sinensis*. Despite heavy and uniform weed pressure in weedy plots, competition had no influence on aboveground biomass, culms m⁻², height or inflorescences m⁻² (Table 2). *Miscanthus* × *giganteus* ‘PowerCane’ (‘Powercane’ hereafter) and our positive control group were taller ($P < 0.001$) and produced more culms m⁻² ($P < 0.001$) than the negative control group (Figure 2). The negative control group did produce 46% and 84% greater aboveground biomass than ‘PowerCane’ and the positive control group respectively. Alternatively, our positive control group produced eight-fold more inflorescence m⁻² than ‘PowerCane’ and our negative controls, which did not differ from one another. *Sorghum bicolor* (negative control), the only annual species in our trial, was taller (244 ± 15 cm) and had significantly greater aboveground biomass (15 ± 6 Mg ha⁻¹) than all other taxa ($P = 0.0014$). *Sorghum halepense* (positive control) produced the greatest number of inflorescences (74.9 ± 6.4 m⁻²) in the first year, while ‘PowerCane’ produced only 1.3 ± 0.2 inflorescences m⁻². None of the 20 accessions evaluated here spread outside the cultivated plot into either adjacent receiving area after one growing season.

Year 2

By 12 months after planting, two culms of *M. sacchariflorus* were observed to have spread into the high competition (Hc) receiving area; no culms were found in the low competition (Lc) receiving area. *Sorghum halepense* spread extensively 0 and 1.5 m into both the Lc (198 ± 18 culms m⁻²) and Hc (152 ± 33 culms m⁻²) receiving areas.

In fall 2012, *M. × giganteus* ‘PowerCane’ was taller (267.5 ± 11.6 cm) and produced more culms (130 ± 22 m⁻²) than negative controls and had greater aboveground biomass (26 ± 3 Mg ha⁻¹) than the positive and negative control groups (Figure 2). Competition treatments influenced biomass, culm number, and height, while disturbance had no effect on any measured parameter (Table 2). However, competition from unmanaged weeds did not reduce ‘PowerCane’ height ($P = 0.1571$), culm number

Table 2. Results of a mixed model ANOVA to evaluate competition and disturbance on aboveground biomass, culm number, number of inflorescences, and height for 20 accessions nested within invasiveness groups observed over three growing seasons in Blacksburg, VA.

		df	Biomass	df	Culm number	Height	Inflorescence number
Year 1	Block	3	0.2267	3	0.1104	0.3410	0.0093
	Species invasiveness	2	<.0001	2	<.0001	<.0001	<.0001
	Species (species invasiveness)	15	<.0001	19	<.0001	<.0001	<.0001
	Competition	1	0.7651	1	0.1297	0.9889	0.5704
	Competition × species invasiveness	2	0.2267	2	0.1482	0.8867	0.9010
Year 2	Block	3	0.3444	3	0.1365	0.4277	0.2615
	Species invasiveness	2	<.0001	2	<.0001	<.0001	<.0001
	Species (species invasiveness)	17	<.0001	17	<.0001	<.0001	<.0001
	Competition	1	0.0475	1	0.0087	0.8145	0.0003
	Disturbance	---	----	1	0.8393	0.5382	0.9390
	Competition × species invasiveness	2	0.0205	1	0.9876	0.0411	0.8763
	Disturbance × species invasiveness	---	----	2	0.9258	0.3361	0.1236
	Competition × disturbance	---	----	1	0.5873	0.8950	0.9295
	Competition × disturbance × species invasiveness	---	----	2	0.9879	0.5629	0.2624
Year 3	Block	3	0.6655	3	0.3943	0.0021	0.5631
	Species invasiveness	2	<.0001	2	<.0001	<.0001	0.0818
	Species (species invasiveness)	16	<.0001	17	<.0001	<.0001	<.0001
	Competition	1	0.2491	1	0.2686	0.3401	0.5298
	Disturbance	1	0.1074	1	0.8797	0.2327	0.1519
	Competition × species invasiveness	2	0.1041	1	0.4864	0.5878	0.5585
	Disturbance × species invasiveness	2	0.0742	2	0.6574	0.7645	0.6781
	Competition × disturbance	1	0.3392	1	0.5440	0.4407	0.8374
	Competition × disturbance × species invasiveness	2	0.8225	2	0.6979	0.4895	0.8232

($P=0.3867$) or biomass ($P=0.2928$). Competition reduced biomass 56% and 70% and culm numbers 26% and 53% of positive and negative control groups, respectively. Interestingly, inflorescence production was not influenced by competition, with positive controls and 'PowerCane', both producing a mean of 62 inflorescences m^{-2} , 95% more than negative controls (Figure 2). *Sorghum halepense* again produced the greatest number of inflorescences ($186 \pm 15 m^{-2}$) among the positive control group, 118% more than naturalized *M. sinensis* PA, which ranked second for inflorescence production.

No further spread of *M. sacchariflorus* was observed between the spring and fall 2012. *Phalaris arundinacea* was observed in the Lc receiving area with 2.7 ± 2.1 culms m^{-2} at a distance of 0 to 1.5 meters from the planted plot. Population density of *S. halepense* continued to increase from year one to year two in the Lc and Hc receiving areas. At the 3 to 4.5 m distance, culm number increased by 584% (from 19 to 130 ± 17 culm m^{-2}) in the Lc receiving area and 420% (from 5 to 26 ± 7 culms m^{-2}) in the Hc receiving area.

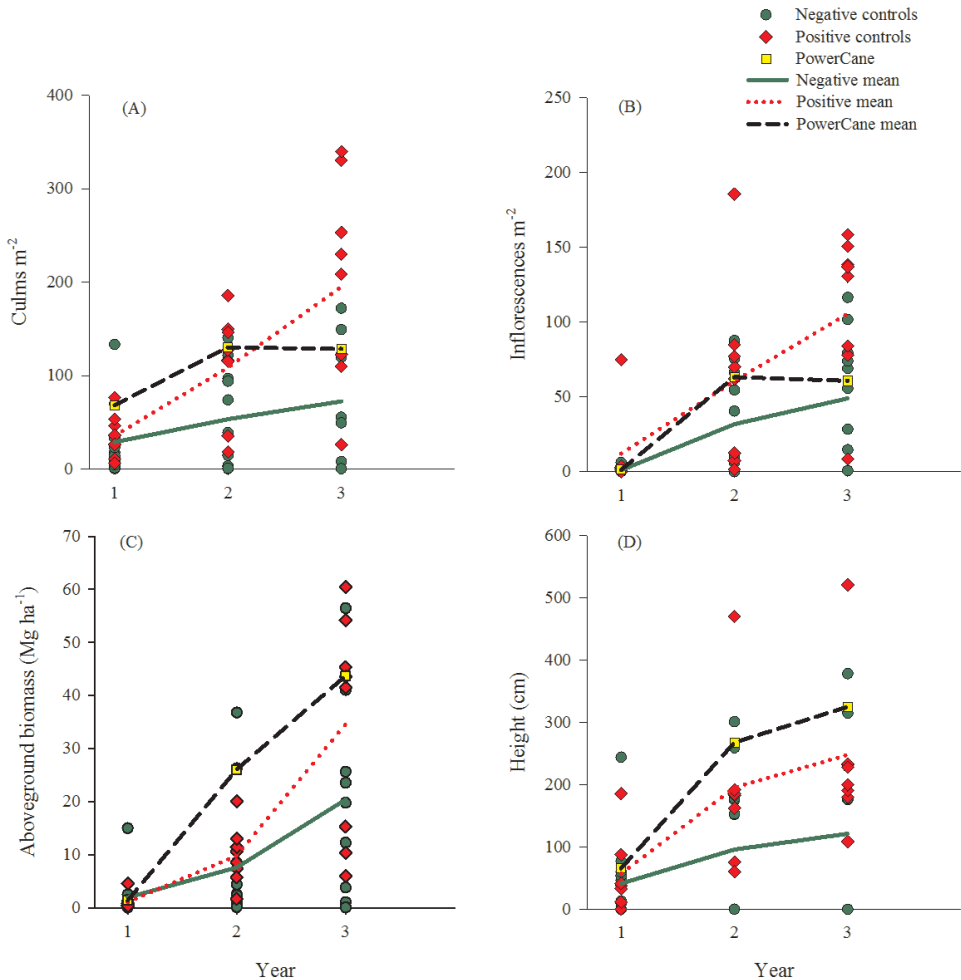


Figure 2. Mean culms (A), inflorescences (B), aboveground biomass (C), and height (D) for 10 species (20 total accessions) observed over three growing seasons in Blacksburg, VA.

Year 3

Local spread of positive control species *S. halepense*, *P. arundinacea*, and *M. sacchariflorus* increased in year three in both Lc and Hc receiving areas. For the first time we observed seedlings of *P. virgatum*, *A. gerardii* and ornamental cultivars of *M. sinensis* (negative controls), ‘PowerCane’, and naturalized accessions of *M. sinensis* (positive controls) outside the cultivated plot in both Lc and Hc receiving areas.

We saw no influence of either competition or disturbance on biomass, culm number, height, or inflorescence number in the third growing season (Table 2). ‘PowerCane’ was taller (325 ± 12 cm) and produced greater aboveground biomass (43.7 ± 6.8 Mg ha⁻¹) than both positive and negative control groups (Figure 2). However, our

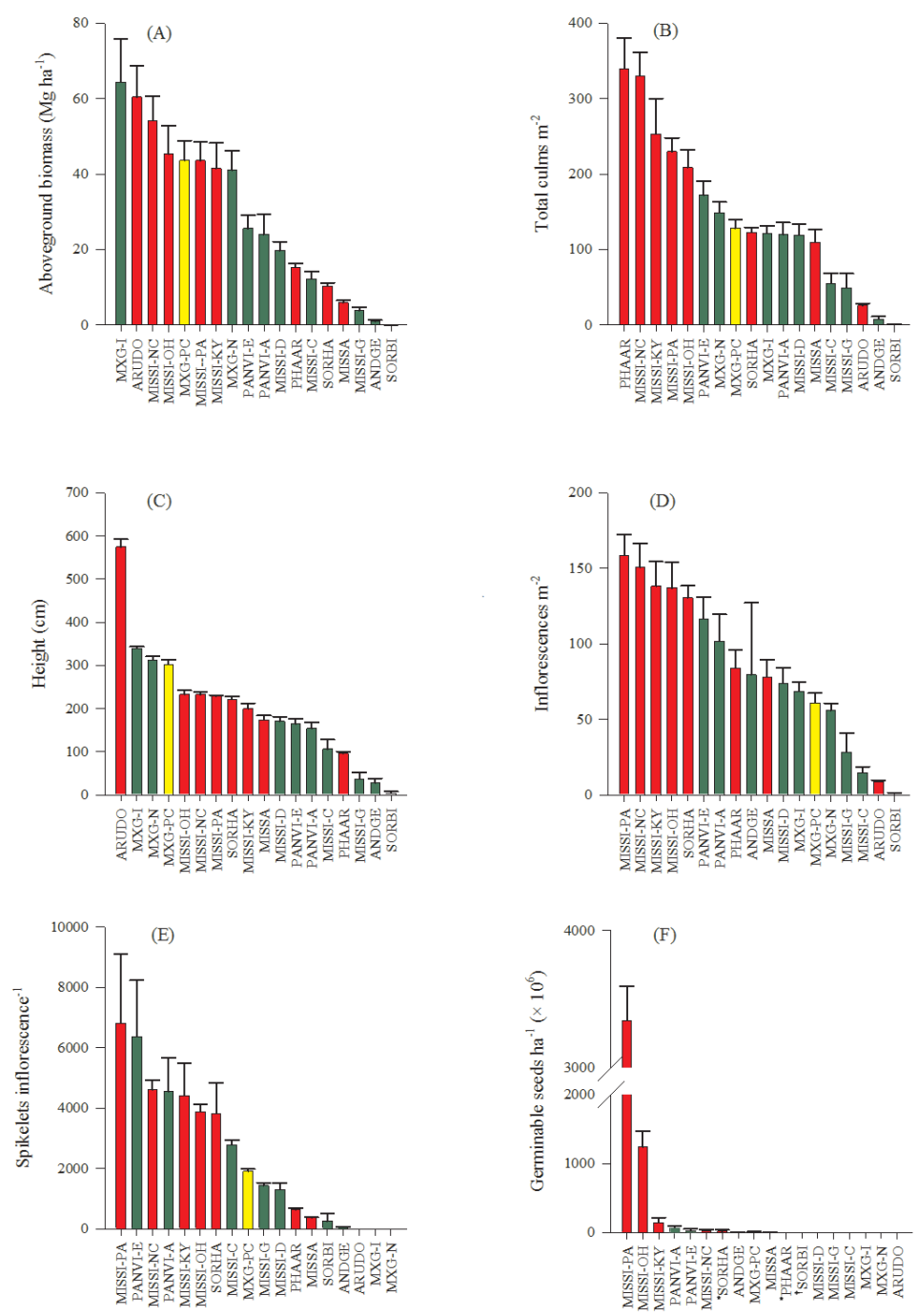


Figure 3. The ranked aboveground biomass (A), culms (B), height (C), inflorescences (D), spikelets (E), and germinable seeds (F) for 18 accessions (two year-old *Saccharum*, spp. were omitted), recorded at the end of the third growing season. Accessions marked with * indicate that seeds appeared immature at time of germination testing, and † indicate that seeds had extreme fungal and insect damage at the time of harvest.

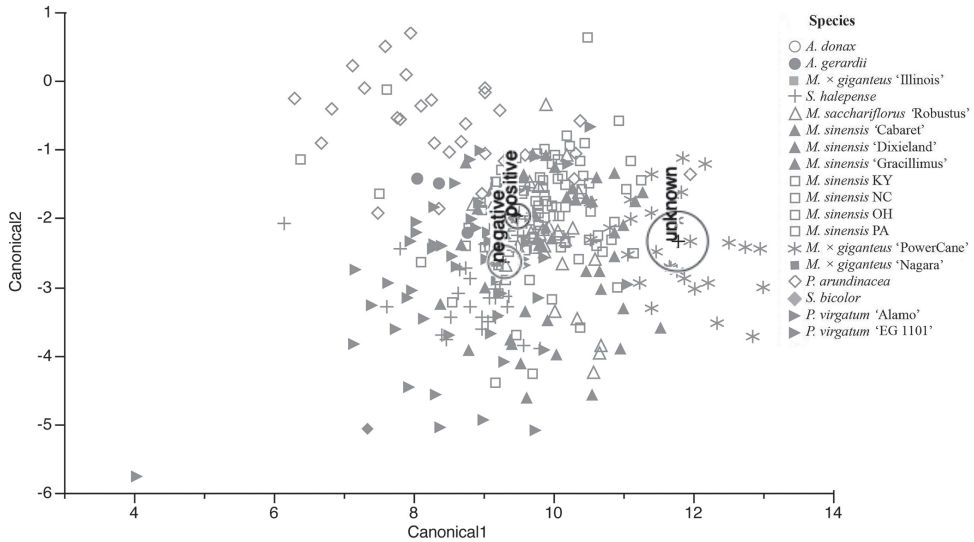


Figure 4. Canonical discriminant analysis plot for the species invasiveness groupings positive controls, negative controls and *M. x giganteus* 'PowerCane'. The fitness parameters biomass, culm and inflorescence number, height and spikelet production were used as predictors.

positive controls produced 52% more culms m^{-2} and 74% more inflorescences m^{-2} than 'PowerCane' (Figure 2). 'PowerCane' produced $2,163 \pm 80$ spikelets inflorescence $^{-1}$, significantly fewer than our positive control group ($3,226 \pm 534$ spikelets inflorescence $^{-1}$) (Figure 3). 'PowerCane' seeds also exhibited low germinability with only one of 300 spikelets germinating after four weeks. Alternatively, two naturalized accessions of *M. sinensis*, PA and OH, had the highest seed germination rates (31% and 16% respectively) (Figure 3). The rank of performance varied among accessions and invasive groups among the functional traits recorded (Figure 3). When our functional traits were used as predictors, canonical discriminant analysis failed to show clustering of our invasive groups, with a predictive value < 62% (Figure 4).

In all cases, seedling or vegetative spread into adjacent receiving areas was greater in the Lc receiving area compared with the Hc receiving area (Figure 5). A significant interaction between invasive groups and receiving area competition was observed at the 0 to 1.5 m ($P < 0.001$) and 1.5 to 3 m ($P = 0.04041$) distances from the cultivated plot (Figure 5). While the greatest number of culms m^{-2} were observed in the 'PowerCane' Lc receiving areas, numbers were not different from those found in the positive control Lc plots. In the Hc receiving areas, culms m^{-2} produced by 'PowerCane' was equivalent to that of the negative control group. It should also be noted that, at no time were any of the sterile clones in this study (*A. donax* or *M. x giganteus* 'Illinois' and 'Nagara') observed to spread vegetatively outside of their associated planted plot.

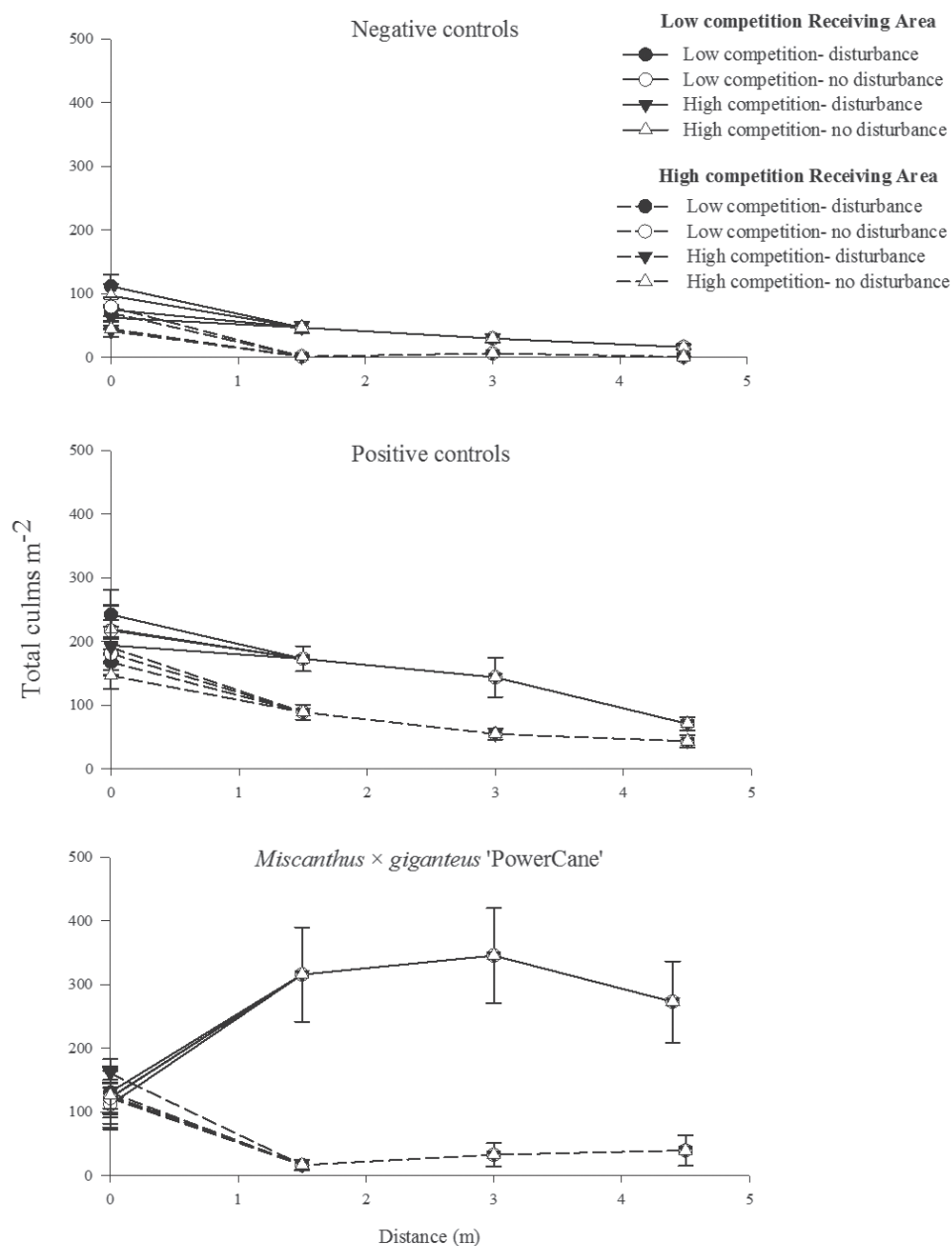


Figure 5. Total number of vegetative and seedling culms m^{-2} observed within the cultivated plot (distance 0 m), and in the Lc, low competition (intensive weed management) and Hc, high competition (no weed management) receiving areas from a distance of 0.1 to 4.5 m from the cultivated plot, after three growing seasons.

Discussion

All taxa in our study established under all treatment conditions, and all fertile crops produced offspring, with the exception of the two *Saccharum* spp., which were only grown for two years. Despite enhanced traits for cold tolerance, these cultivars may have been well beyond their suitable geographic range in Blacksburg, VA (Barney and DiTomaso 2011) because they were the only species in this study to decrease in biomass (1300% Mg ha⁻¹) and culm number (380%), as well as fail to produce inflorescences; however, the lack of inflorescences production was not unexpected as several abiotic factors are responsible for inhibiting and inducing tasseling, such as temperature and photoperiodism (LaBorde 2007).

Though all of the perennial species in our study have the ability to spread vegetatively, local spread was equivocal. Despite the three year clonal expansion of sterile *M. × giganteus* 'Illinois' (0.23 m² plant⁻¹ increase in area) and *A. donax* (0.28 m² plant⁻¹ increase in area), this did not contribute to nascent plants outside the cultivated plot. Unlike the culms of most species in our study, which die back at the end of each growing season, the culms of *A. donax* remain dormant during the winter months (Saltonstall et al. 2010). *Arundo donax* produced 33 ± 1 nodes culm⁻¹; the majority of which produced new axial shoots each growing season. Despite overwhelming evidence of *A. donax* clonal spread in warm riparian or coastal freshwaters of the southwestern United States (Bell 1997; Quinn and Holt 2008; Seawright et al. 2009); the numerous culms we observed, bending to the ground at the perimeter of our plots, failed to root and produce new ramets; so called layering (Boland 2006). It has been suggested that the probability of a plant becoming invasive increases with the ability to reproduce vegetatively (Kolar and Lodge 2001). The only species in our trial for which vegetative reproduction appeared to contribute to invasive spread were *S. halepense*, *M. sacchariflorus*, and *P. arundinacea* (all positive controls), all of which began to spread from the planted plots in the second growing season. Conversely, Pyšek and Richardson (2007) argue that while vegetative traits may benefit persistence, the ability to spread may be hindered by vegetative reproduction, especially if seed production is limited or absent. In agreement with our results, all of the caespitose grasses in our study failed to spread vegetatively beyond the borders of the planted plot under our growing conditions.

The production of fertile seed enhanced the ability of many species to spread, but only locally. *Sorghum halepense* was the only accession to have large numbers of first season inflorescences (Figure 2B), and so it is not surprising that this was the only species to generate seedling volunteers in the second year. In contrast, most taxa increased inflorescence production in the second season, extending the range of species detected as seedlings in the receiving areas in the third season. With the exception of 'PowerCane', inflorescence numbers continued to increase from the second to the third growing season, suggesting that propagule pressure in the receiving plots did not reach a plateau during the experiment. While other factors such as habitat invasibility and timing are essential to invasive success (Barney and Whitlow 2008), this dramatic increase in propagule pressure facilitated establishment of 'PowerCane' seedlings in

both the Lc and Hc receiving area, though recruitment varied dramatically between the habitats. Inflorescence production and therefore the total number of spikelets plot⁻¹ in our three invasive groups increased between year one and year three. Even small increases in propagule pressure can result in a substantial increase in invasion pressure even in inhospitable environments (Davis 2009), which likely contributed to the observed lag.

The more individuals released into an environment the higher the probability that some propagules will endure environmental barriers and overcome stochastic biotic and abiotic factors (Blackburn et al. 2009). The number of introduced individuals, therefore, has a substantial influence on establishment success (Lockwood et al. 2009). Seeds of *A. gerardii*, *P. virgatum* and *P. arundinacea* are known to have variable dormancy and potentially low seedling vigor (Beckman et al. 1993; Lewandowski et al. 2003; Parrish and Fike 2005; Smart et al. 2003), which agreed with our observations in the establishment year. *Miscanthus sacchariflorus* (positive control) has been reported to have low seed set, ~746 viable seeds mature plant⁻¹, with population growth predominantly due to vegetative spread (Madeja et al. 2012). Despite these assumptions, 1% of tested *M. sacchariflorus* seeds did germinate, which was greater than seed germinability of 'PowerCane' (0.3%) and *M. sinensis* NC (0.6%). Previous research has shown ornamental cultivars of *M. sinensis* are extremely variable, ranging from 190,000 seeds plant⁻¹ to 3,100 in 'Gracillimus', 785 in 'Dixieland' and 0 filled spikelets plant⁻¹ in 'Cabaret' (negative controls) (Madeja et al. 2012; Meyer and Tchida 1999). Results from our seed germination testing support these conclusions in which no ornamental *M. sinensis* seeds germinated; however, we observed high variability (31% to 0.6%) in germinability for naturalized *M. sinensis* accessions (Figure 3). Dougherty et al. (2014) showed that ~44% of seed from weedy accessions of *M. sinensis* germinated in a laboratory setting. Previous research indicates that both inflorescence and spikelet production in almost all *M. sinensis* cultivars are positively correlated with plant hardiness zone (Madeja et al. 2012; Meyer and Tchida 1999; Wilson and Knox 2006); indicating that invasion pressure for these species may vary with latitude.

After three years of growth, all naturalized accessions of *M. sinensis* produced a greater number of inflorescences m⁻² and more spikelets inflorescence⁻¹ than 'PowerCane' (Figure 3). In this study, 'PowerCane' produced ~1.3 billion spikelets ha⁻¹ in the third growing season, which would yield 3.9 million viable seed ha⁻¹ given the 0.3% germination rate. It is possible that we saw greater numbers of seed produced than in a production field; like *P. virgatum* (Martinez-Reyna and Vogel 2002), *Miscanthus* spp. are self-incompatible (Hirayoshi et al. 1955). The genetic diversity among the accessions and species in our study, ideal for outcrossing species, may have led to inflated seed production (Madeja et al. 2012) compared to the relative genetic homogeneity common in commercial crops. In this case genetic variability not only increases the likelihood of seed production, but also has the potential to enhance establishment success and increase the habitat range of exotic species (Lockwood et al. 2005).

Surprisingly, none of the accessions were affected by the level of competition, which not only contradicts much of the literature suggesting the need for weed man-

agement at establishment (Lewandowski et al. 2003), but supports the conclusion that these species are fast growing and highly competitive (Raghu et al. 2006). The high seedling establishment of ‘PowerCane’ in the Lc and lower seedling establishment numbers in the Hc, appears to contradict our recent findings in which only 0.1% of emerged ‘PowerCane’ seedlings survived after six months (Smith and Barney 2014b). However, our earlier study indicated the majority of ‘PowerCane’ seedlings emerged in areas of available bare ground and low resident plant competition such as agricultural fields and forest understories. However, annual weed species dominated our Hc receiving area, which created open spaces and the availability of bare ground during the winter months and early spring, coinciding with annual seed dispersal for many of these late flowering species. The small, light seed and ciliate lemma (Gleason 1952) of *Miscanthus* spp. is valuable for dispersal. However, the ciliate lemmas appear to interfere with the ability of *Miscanthus* seed to make important soil contact required for germination, which is evident in areas of high resident plant competition and litter (Smith and Barney 2014b). According to Quinn et al. (2011), the anemochorous *M. sinensis* spikelets have been shown to disperse an average of 50 m. Therefore, open areas of low resident plant competition near production sites will likely be the most susceptible, suggesting that our Lc plots represent a worst case scenario. This information will be critical for identifying susceptible habitats near cultivated fields and will be important considerations for management.

Despite the utility of trait-based research for helping to make associations and guide management, traits do not confer absolute predictability. Invasions will always be contingent on a number of interacting factors (Barney et al. 2008; Dredovsky et al. 2012). Hence, our experimental design was critical in the interpretation of our results. Clearly this geographic location, habitat and treatment factors were ideal for a species such as *S. halepense*; a species intentionally introduced for agronomic purposes, which now flourishes in agricultural and anthropogenic systems of the southeastern U.S. (Warwick and Black 1983). However, despite the reputation of *A. donax* as an aggressive riparian invader, this species appeared to be constrained by the non-riparian landscape.

The selection of our invasive and noninvasive taxa was a novel methodology used to make important relative comparisons. The ten species selected in this study are of similar life form, and all of them, including *S. halepense* (Nackley et al. 2013), have the potential to be used as a feedstock in bioenergy production. Comparisons of ‘PowerCane’ to specific taxa, *S. halepense* for example, across an invasive spectrum, provided meaningful information. However, making all pairwise comparisons would not be ecologically and statistically meaningful; therefore, our goal was to also find and examine broader trends within invasive groups. Interestingly, our positive control species were selected on the basis of a past history of invasiveness, a robust predictor of future invasiveness (Davis 2009). However, multivariate analysis failed to indicate a relationship between our measured functional traits and our invasive groups (Figure 4). After three growing seasons, our ranking of fitness metrics also fails to show the anticipated consistent gradient of positive (high fitness) to negative (low fitness). This suggests that functional traits vary in their relationship to invasiveness (Dredovsky et al. 2012).

However, meta-analysis reveals that traits such as fitness, size, and growth rate were significantly higher for exotic invasive species when compared with the traits values of non-invasive species (van Kleunen et al. 2010). It is imperative to remember that invasiveness is not a “one size fits all” scenario, which is made evident in our study. Traits vary with life stage and environmental conditions and the importance of any given trait will therefore also vary (Davis 2009; Pyšek and Richardson 2007). While our positive and negative invasive groups may not have clustered as we hypothesized; the use of numerous species and accessions allows us to evaluate the usefulness of traits across an invasive spectrum.

Unfortunately the loss of our Kentucky and Alabama sites limits our ability to generalize across a broader geographic range. The ability to observe relative comparisons across a gradient of species reinforces the fact that it is the important interaction of species and habitat that result in invasive populations (Barney and Whitlow 2008). Our results indicate that several candidate feedstocks have the ability to move from the cultivated field, but it should be noted that we only recorded spread to < 5 m from the field edge in three years. On average, more than 100 culms m⁻² were observed in every measured quadrat in the Lc receiving area of *S. halepense*, naturalized *M. sinensis*, *P. virgatum* and ‘PowerCane’. Conversely, only *S. halepense* and naturalized *M. sinensis* maintained high numbers in the Hc receiving area when *P. virgatum* and *M. × giganteus* ‘PowerCane’ showed a dramatic decrease in culm numbers.

Conclusion

Bioenergy crop movement beyond the cultivated field would not be novel to agronomic crops because feral escapes are known for most row crops (DiTomaso and Healey 2007). Yet due to their economic and social importance, crops are not frequently discussed in the invasion literature. Our experiment covers the first three years of the establishment phase, and unexpected and nonlinear changes may manifest in subsequent stages. Our results suggest that at least short-range movement away from the cultivated field is probable for fertile bioenergy feedstocks. It should be noted that no species in this study were detected outside our trial boundary, which predominately consisted of a mowed perennial border. Further study, across broader geographic locations and continued research will be necessary to further determine acceptable risk and management planning.

The use of ‘PowerCane’ or other fertile *M. × giganteus* germplasm could improve grower adoption but the invasive potential and ecosystem impacts of widespread cultivation still require further evaluation including the determination of climatic limitations of *M. × giganteus* and other bioenergy crop seedlings. The ability to contextualize our results suggests that *M. × giganteus* ‘PowerCane’ did not have the highly competitive seedling establishment potential of *S. halepense*. Alternatively, in this growing region sterile cultivars provide a lower risk option, but require additional economic investment. The scrutiny that has been applied to bioenergy crops indicates that we

have moved beyond the once cavalier approach toward species introduction. These efforts should continue in order to reduce unwanted and unintentional invasive spread. Nascent populations or seedlings may be easily overlooked. However, management at the seedling or early growth stage will likely increase the chances of successful control (Stephens and Sutherland 1999). Our relative methodology and results from this study can help us prepare for industry development while helping to minimize risk and mitigate invasive spread.

Acknowledgments

Thanks to Ryan Dougherty, Eugene Dollete, John Halcomb, Matt Ho, Daniel Tekiela, Elise Benhase, Phillip Cox, and Carissa Ervine for help during installation, data collection, and harvest of this experiment. Thanks to Mendel Biotechnology, Inc for donation of *M. × giganteus* ‘PowerCane’ and ‘Nagara’ plugs and Dr. Erik Sacks for naturalized *M. sinensis* seed. The USDA graciously provided the two *Saccharum* spp.

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Missing the bandwagon: Nonnative species impacts still concern managers

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Academic editor: L. Foxcroft | Received 10 November 2014 | Accepted 19 February 2015 | Published 14 April 2015

Citation: Kuebbing SE, Simberloff D (2015) Missing the bandwagon: Nonnative species impacts still concern managers. NeoBiota 25: 73–86. doi: 10.3897/neobiota.25.8921

Abstract

Recent critics of invasion biology advocate reduced efforts against nonnative species, arguing that attempts to manage invasions are often costly and futile and that managers are indiscriminately managing species regardless of their impact. Whether this criticism has affected ground-level operations is unknown. A survey of land stewards of a major conservation NGO reveals that this plea has not been heeded; in fact, managers report that they would increase nonnative species management if more resources were available. While respondents overwhelmingly listed nonnative, invasive species as their highest priority for management, we found little evidence supporting the criticism that practitioners focus on nonnative species with minimal ecological impact. Additionally, we found that more conservation practitioners reported managing problematic “weedy” native species over nonnative species that are not invasive. Our results indicate that these managers are selective rather than profligate, targeting species that are having a demonstrable impact or are likely to do so. They feel hamstrung by resource shortages despite their rigorous target selection. Why the increasing literature calling for changed treatment of nonnatives has not led to changed operations is uncertain. Possible reasons are that the critics are not prescriptive or unified about management prioritization, or that these managers simply reject the proposed new paradigm.

Keywords

New conservation science, nonnative species, nonnative invasive species, management

Introduction

The past decade has seen vocal critics of invasion biology and management (Davis 2009, Davis et al. 2011, Valéry et al. 2013, Thompson 2014) argue that attempting to redress invasions is often costly and futile. A recurring criticism of management programs is that too much time and money are expended managing nonnatives that have minimal impact and that managers' should focus on those species with the largest impact, regardless of the species' origin. However, most critics agree that species—native or nonnative—that negatively affect ecosystems should be managed. Complaints against invasion biology and management vary, but our primary concern here is the argument that management is wasteful and that managers are indiscriminately managing species regardless of their impact.

This barrage of criticisms raises concern that policymakers and managers could lessen or even stop successful (Simberloff 2013) but frequently costly, arduous campaigns to counter invasions (Simberloff and Vitule 2014). There is precedent (e.g., the anthropogenic climate change “debate”) for policymakers to use scientific “controversies” to stop or delay environmental measures (Oreskes and Conway 2010). The increase in both academic publications (Davis 2009, Davis et al. 2011, Valéry et al. 2013, Thompson 2014) and public media attention (Chew and Carroll 2011, Zimmer 2011, Marris 2013) warrants concern that public support for invasive species management may decrease.

These criticisms of invasive species management distill into two major suggested revisions of current invasive species management practices: first, that managers should distinguish between nonnative species that are invasive and those that are not (Davis 2009, Davis et al. 2011, Chew and Carroll 2011), and second, that managers should be concerned with native problematic species as much as nonnative invasive species (Warren 2007, Davis 2009, Davis et al. 2011, Shackelford et al. 2013). A frequent response to these management critiques is that they are straw man arguments because managers already prioritize among nonnative species to target those deemed harmful to specific conservation goals (Simberloff et al. 2011, Richardson and Ricciardi 2013). This debate over current management practices has stagnated, in part, because it is unknown whether these calls to change management paradigms have any influence on ground-level management philosophies or activities.

The call to de-emphasize management of nonnative species is related to a larger crusade that invokes an even stronger call to revise “traditional” conservation management practices. In the past decade, the “new conservation science” (NCS) movement has sought to refocus conservation biology on protecting nature for services it provides to humans rather than on saving nature for its own sake (Doak et al. 2014). NCS advocates often argue that traditional conservation is inadequate for preserving biodiversity and that some degrading natural forces (such as nonnative species) are so powerful and inexorable that certain strategies and goals of traditional conservation should simply be abandoned. This view has led to heralding of a “new world order” (Hobbs et al. 2006, 2009) in which restoration ecologists will abandon the

goal of returning an ecosystem to a semblance of its historical reference condition. In this new world, removing or managing nonnatives is futile after ecosystems pass an irreversible “restoration threshold” to become “novel ecosystems” (Hobbs et al. 2009). Instead, its advocates seek to harness “novel ecosystems” that arise because of invasions and global climate change to produce services to humankind (Hobbs et al. 2009, Lalasz 2011, Schlaepfer et al. 2011). Again, results of calls to reform nonnative species management practices in response to these broader conservation paradigm shifts are unknown.

To address how managers are selecting and prioritizing species for management on conservation properties, we surveyed land stewards from one of the largest international conservation organizations. The Nature Conservancy (TNC), one of the largest landowners in the United States, owns approximately 6 million hectares and assists in management decisions for tens of thousands more hectares (TNC, www.nature.org). In previous IUCN threat classification assessments, “Invasive & Other Problematic Species” were listed as one of the greatest threats to conservation priorities for TNC conservation projects (Kuebbing et al. 2012). Conservation managers at TNC provide an ideal group for testing how recent debates on nonnative species management philosophy have affected on-the-ground operations because recent critiques of current invasive management programs have garnered an upswell of responses from TNC staff (Chadderton 2011, Gordon 2011, Jacquart 2011, Serbesoff-King 2011, Zimmerman 2011) and its Chief Scientist (Kareiva 2011, Kareiva et al. 2011, Kareiva in Dunkel 2011, Lalasz 2011, Kareiva and Marvier 2012, Voosen 2012), which indicates that the organization is actively debating the role of nonnative species management within its larger conservation mission. Additionally, because of the large amount of land owned by TNC, it employs many conservation practitioners who are actively making management decisions for conservation.

Here, we are interested in the attitudes and on-the-ground actions of TNC staff who manage TNC preserves. We queried TNC land stewards and support staff on their attitudes about nonnative species, management of them, and specifically how they prioritize management activities among species, whether native and nonnative as well as invasive or not invasive. We asked to what extent a drumbeat of academic publications (Davis et al. 2001, Hobbs et al. 2009, Valéry et al. 2013) and publications and talks by TNC’s Chief Scientist (e.g., Kareiva et al. 2011, Kareiva in Dunkel 2011, Kareiva and Marvier 2012) has changed TNC management activities.

Materials and methods

We created an online survey (Suppl. material 1) containing a mixture of closed-ended questions, including yes/no, Likert scale, check-all-that-apply, and ranked response options. Many questions included an “other” option for open-ended responses. Options for check-all-that-apply and ranked response questions were randomized to avoid effects of option order on answers. The survey was divided into five sections:

impacts, management, selection of species for management, TNC Global Invasive Species Team (GIST), and debate on nonnative species impacts. Although the GIST was dismantled in 2009 owing to budget cuts, we included questions about it in the survey because this resource was a widely-used aggregation of resources and communication pathway for TNC employees making decisions regarding nonnative species (Serbesoff-King 2011). For those TNC employees whose tenure overlapped with the GIST, we can gauge how managers have adapted to finding information on nonnative species management and whether their assessment of nonnative species management needs have changed, based on whether they feel the need for the GIST to be resurrected. For questions designed to determine whether managers distinguish between nonnative species and invasive nonnative species, we defined each species type (Suppl. material 2). Our software allowed us to move respondents through the survey depending upon answers (e.g., a respondent who did not manage nonnatives skipped all management questions). Each respondent answered between 10 and 18 questions.

We emailed 206 employees through TNC's "Stewardship" internal listserv. This listserv represents employees who manage conservation lands owned or monitored by TNC and employees who provide research and guidance on management. We emailed the survey on February 24, 2014 and sent a reminder on March 31, 2014.

Results

Respondent demographics

Eighty-eight employees – 43% of the total listserv – completed the survey. Over three-quarters of respondents were highly involved in nonnative species management decisions, either sharing decision-making (60%) or making decisions themselves (17%). Thirteen percent made some decisions, and 6% had no substantial management input. Many respondents have worked over 10 years for TNC (39%), and nearly one-quarter 6–10 years. Most respondents had a college or university bachelor's (45%) or master's degree (47%).

Impacts of nonnative species

Nearly all respondents thought a proportion of nonnative species caused ecological or aesthetic impact (Figure 1a–d). For all impacts, the majority of managers felt only "some" (defined in the survey as < 25%) nonnative species caused impact, and managers were unlikely to report that they believed "all" (defined in the survey as 100%) nonnative species caused impact. Likewise, very few managers described nonnative species as having "no (0%)" impact (Figure 1).

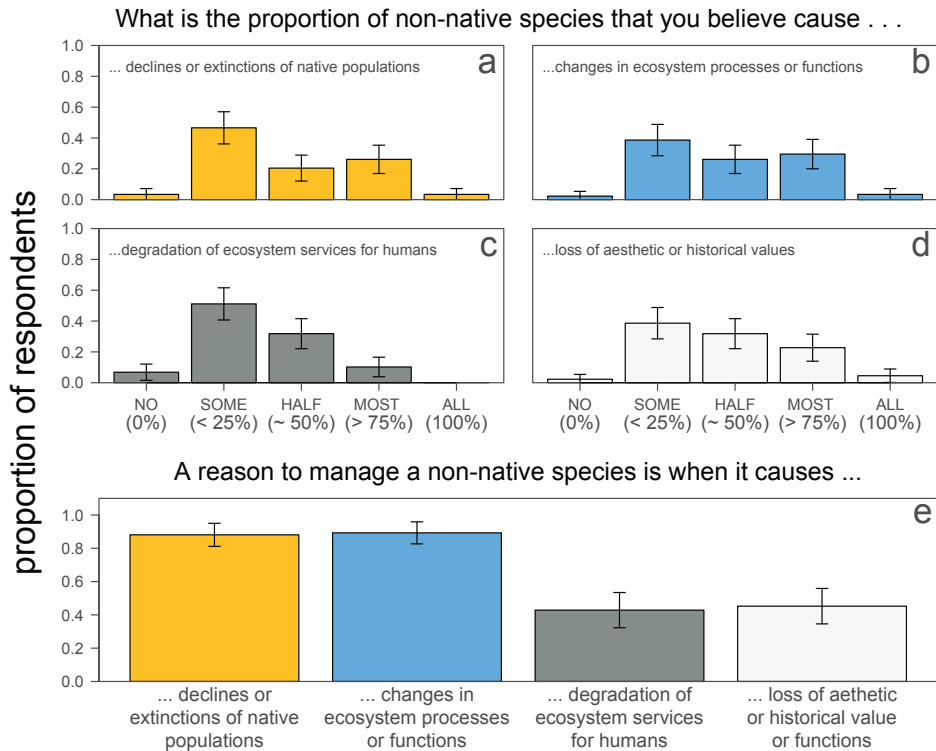


Figure 1. Impacts of nonnative species. Managers thought a lower proportion of nonnative species were likely to degrade ecosystem services for humans (**c** Fisher's Exact Test, $P < 0.05$) compared to other ecological or aesthetic impacts (**a, b, d**), and nonnative species impacts on native species populations or ecosystem processes were more likely to be cited as justification for managing them (**e** Fisher Exact Test, $P < 0.001$). Error bars represent 95% confidence intervals.

Management of nonnative species

Almost all respondents managed nonnative species (95%). Of respondents who did not currently manage them (5%), three reported they had managed nonnatives on TNC properties in the past but no longer do so because other goals have higher priority.

Ecological impacts of nonnative species motivated management for respondents who managed for nonnative species (Figure 1e). Respondents were more likely to manage nonnatives because they believe they cause changes in ecosystem processes or functions or declines or extinctions of native species populations than because nonnatives decreased aesthetic or historical value or degraded ecosystem services for humans (Fisher Exact Test, $P < 0.0001$). Some respondents provided additional reasons for managing nonnatives, including additional ecological impacts (e.g., nonnatives changing the condition of rare ecological communities or ecosystems, promoting other nonnative spe-

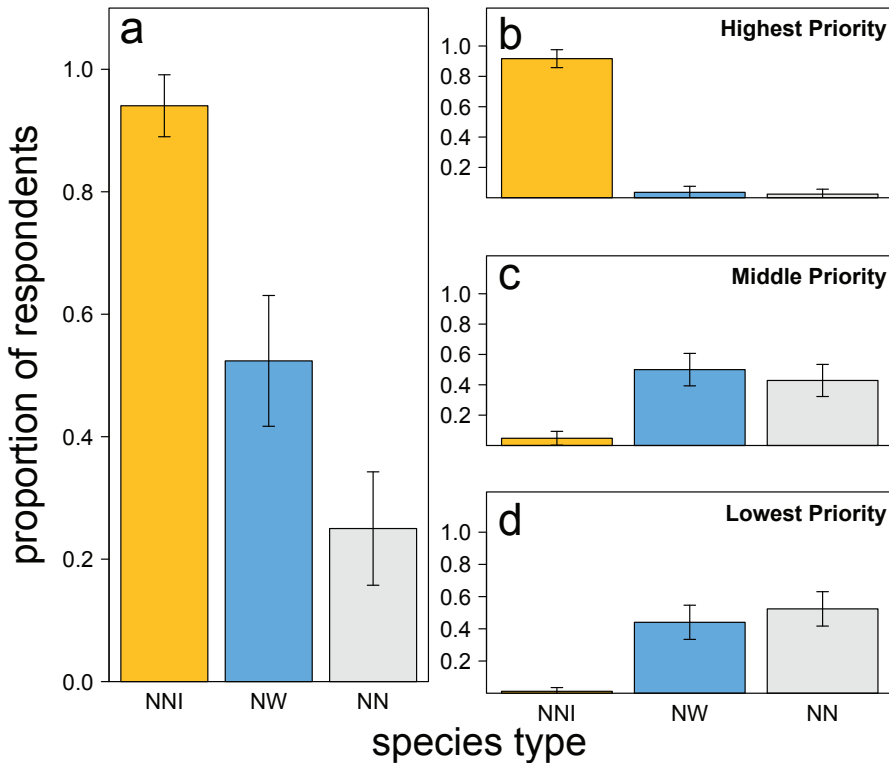


Figure 2. Selection of species for management. Managers were most likely to report that they managed nonnative invasive species (NNI, gold **a**) on conservation properties, and managers ranked NNI as the highest priority for management (**b**) compared to native weedy species (NW, blue) or nonnative species that are not invasive (NN, gray). Error bars represent 95% confidence intervals.

cies, or decreasing ecological resilience), philosophical grounds (e.g., the “precautionary principle”), or contractual requirements (e.g., in-lieu fee mitigation program).

Most respondents who managed for nonnative species allocated less than half their resources (time, money, labor, etc.) to management, but nearly all reported they would increase allocation if more resources were available (Figure 4). Approximately half of the managers reported resources for nonnative species management increased, and one-quarter reported decreases. Over the past 5–10 years, one-quarter of respondents have had stable resource allocation (Figure 4).

Selection of species for management

Nonnative invasive species were the most frequently reported management target (Figure 2a), and 92% of managers ranked nonnative invasive species as the highest priority (Figure 2b). Managers were twice as likely to report they managed native weedy species than that they managed nonnative species that are not invasive

(Figure 2a). Native weedy species and nonnative species that are not invasive were equally likely to be ranked as middle and lowest priority (Figure 2c, d; Fisher's Exact Test, $P > 0.1$).

TNC Global Invasive Species Team (GIST)

Approximately half of the stewards worked for TNC when the GIST was operating, and of these respondents nearly all reported that over 75% of their interactions with the GIST had aided their nonnative species management activities. Two-thirds of respondents familiar with the GIST indicated that it would aid their management activities if TNC revived the GIST, and the remaining one-third were "not sure" whether reviving the GIST would be helpful. Currently, respondents find information on nonnative species management from colleagues who manage nonnatives, other websites (e.g., Global Invasive Species Database, Center for Invasive Species and Ecosystem Health, Center for Invasive Species Management, Delivering Alien Invasive Species Inventories for Europe, etc.), and the primary literature (i.e., peer-reviewed publications).

Debate on the impact of nonnative species

Almost all managers agreed that nonnative species often cause ecological problems and should be of ecological concern (Figure 3b) and disagreed that people who think nonnatives are often a problem are xenophobic (Figure 3a). However, managers were split on whether "native weedy species cause ecological problems as much as nonnative species" (Figure 3e). Approximately one-quarter of respondents agreed and one-third disagreed with the sentiment that native weedy species and nonnative species were equally likely to cause ecological impacts. Respondents more frequently cited personal (74%,) or peer (60%) knowledge and first-hand experiences as more influential than academic research and journal publications focusing on the current nonnative species management debate (34%) in affecting their opinions about nonnatives.

Discussion

Our survey shows that nonnative species management remains an important component of conservation strategies at The Nature Conservancy. We found little evidence supporting the recent criticisms that managers do not distinguish between "nonnative" and "nonnative invasive" species, or that managers are more concerned with nonnative species over problematic native species. Managers responsible for protecting conservation properties believed a proportion of nonnative species cause ecological impact and reported that managing nonnatives species that cause these impact was justified. Generally, respondents dedicated a sizeable fraction of resources to nonnative manage-

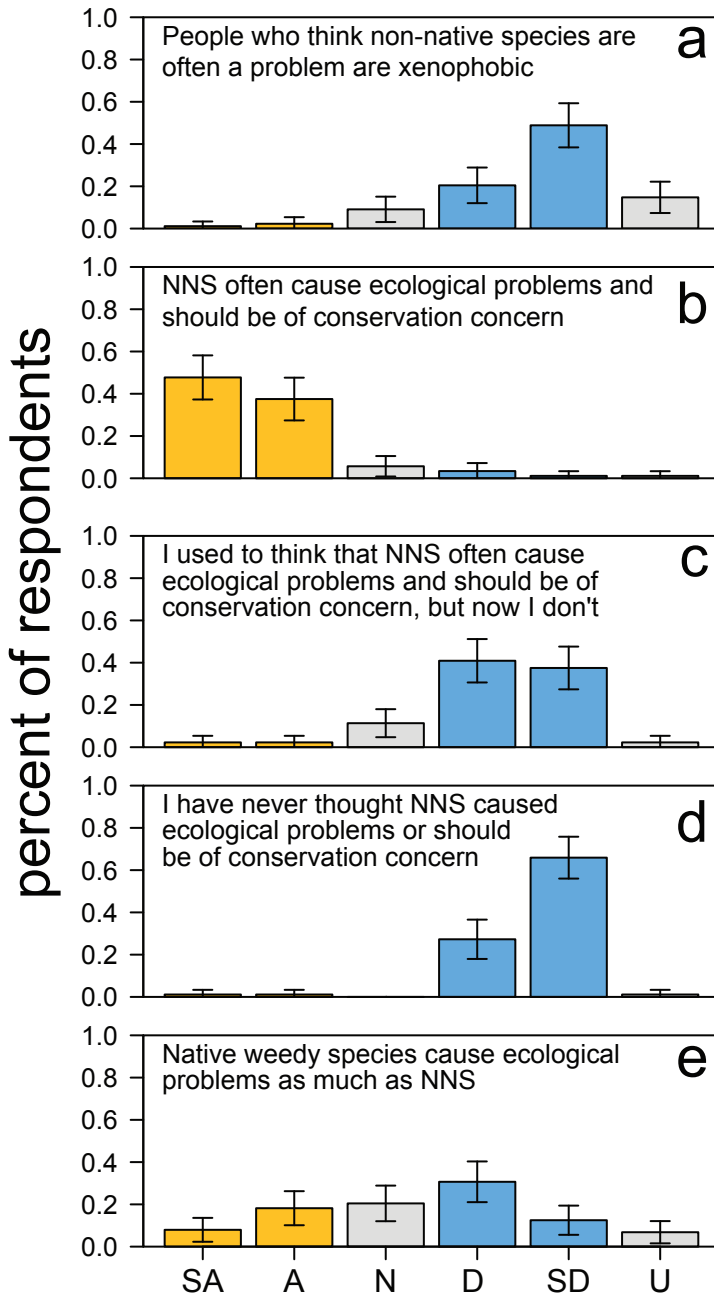


Figure 3. Debate on the impact of nonnative species. Managers rank how strongly they agree or disagree with the following statements regarding non-native species (NNS) management. Abbreviations represent the following: SA = strongly agree, A = agree, N = neutral, D = disagree, SD = strongly disagree, U = uncertain.

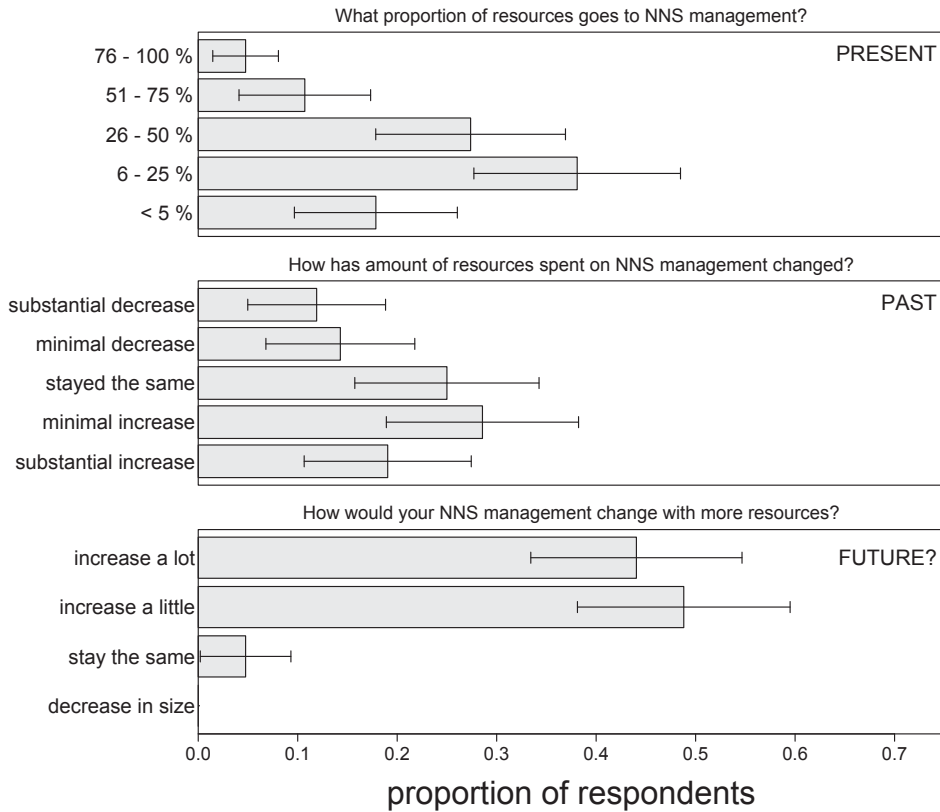


Figure 4. Resource allocation for species management. The majority of respondents who managed for non-native species (NNS) allocated less than half their total resources (time, money, labor, etc.) to management and have seen minimal to no change in resource expenditure in the past 5–10 years. Nearly all respondents would increase non-native species management if more resources were available. Error bars represent 95% confidence intervals.

ment activities, but nearly all respondents would increase such management if given more resources. Most managers reported their motivations for nonnative species management were based upon personal and peer first-hand experiences and not academic journal publications. This may explain why, despite the flurry of prominent academic publications calling for the “end of invasion biology” or fundamental shifts in management strategies, TNC managers have not adopted a new *modus operandi* with respect to nonnative species.

Although managers spend a sizeable amount of resources managing nonnatives, we detected no indications of profligacy. Respondents indicated that they lacked the resources necessary to manage nonnative species adequately, which suggests they are not likely to be wasteful of the limited resources currently available. Though critics suggest that managers “vilify” *all* nonnatives (Davis 2009, Davis et al. 2011, Chew and Carroll 2011), we found evidence that managers are scientifically savvy and

thoughtful in their definition of what makes a species “problematic” and in their management approach. For example, less than 5% of respondents indicated that “all (100%)” nonnatives have impact, and 95% of respondents ranked “nonnative invasive” species rather than all nonnative species as the highest management priority. This indicates an ecological “triage” for managing nonnatives with perceived impact. Perhaps there is a nuanced distinction in how managers target species; managers must prioritize based on “realized” impact (i.e., a widespread species on a property) and “possible” impact (i.e., a species with known impact elsewhere), but if managing a species is inexpensive (i.e., new to an area) and has high possible impact, they should manage for it even if it has not been found “guilty” on their preserve (Pyšek and Richardson 2010, Helzer 2011, Zimmerman 2011). These results also suggest that perhaps the best academic course of action is to continue studying nonnative impacts and prioritization tools to aid species prioritization.

Respondents seem skeptical about academic research, and the invasion management controversy in particular, with respect to their actions on the ground. They were more likely to value personal and peer knowledge and first-hand management experiences than academic research and journal publications. This finding concurs with recent surveys concerning the “knowing-doing” gap in invasive species management, which show that limited access to scientific publications, limited time to peruse literature, and limited overlap in utility of theoretical research to applied issues contribute to infrequent reliance on academic publications for management (Matzek et al. 2014).

The tenet of both the “novel ecosystem” (Hobbs 2006, Hobbs et al. 2009) and the “New Conservation Science” (NCS) movements (Lalasz 2011, Schlaepfer et al. 2011) is that managers should prioritize all management strategies, including dealing with nonnative species, around the concept of “ecosystem services for humans” has not carried the day among TNC managers. Although 41% of respondents listed impact on ecosystem services as a motive for managing nonnatives, this was nearly equivalent to the number listing changes to aesthetic or historical value (43%) and far less than those listing impacts on native species (84%) or ecosystem processes (85%) that may or may not directly affect ecosystem services to humans. This may be a result of the overarching mission of TNC (i.e., “to conserve the lands and waters on which all life depends”), which dictates priority to land protection and all forms of biodiversity (not just humans). Additional possible reasons for TNC managers’ focus on native species and ecosystem processes could be a research bias (i.e., fewer studies on ecosystem service impacts), or that nonnatives are less likely to cause these impacts, or simply that this focus reflects managers’ personal preferences. However, because ecosystem processes are intimately connected to several ecosystem services (Millennium Ecosystem Assessment [MEA] 2005), the substantial number listing ecosystem processes could have been at least partly motivated by concern for ecosystem services, but the relevant survey question listed ecosystem processes and ecosystem services separately and permitted positive responses to both. Certainly there is no doubt that nonnative species can greatly affect ecosystems services, both negatively (e.g. bush honeysuckle, *Lonicera*

spp., increasing human Lyme disease risks, Allan et al. 2010) and positively (e.g., pollination services by nonnative European honeybees, *Apis mellifera*, Potts et al. 2010). Although there has been an effort to frame nonnative species impacts in terms of ecosystem services (Vilà et al. 2010), perhaps invasion biologists should be more explicit about how nonnative species impacts fit into the MEA framework.

Although the overall tone of those seeking a new paradigm for invasion management can only suggest a deemphasis of the entire project, there is a mixed message. For example, even the most vocal critics agree that preventative measures, such as policies that reduce introductions of new nonnatives or early detection and rapid response measures for small invasions, should be promoted and prioritized within conservation programs (Chew and Carroll 2011, Davis et al. 2011, Lalasz 2011). Additionally, many critics concur that the management of widespread nonnative species is justifiable when there is “good” evidence that those species are impacting native species or ecosystems. Thus, the critiques regarding current nonnative species management programs boil down to whether managers indiscriminately target nonnative species regardless of impact—an allegation that these data strongly reject—or that conservation managers and the critics disagree on what constitutes “good” evidence for justifying the management of particular nonnative species.

Conclusion

The management of nonnative, invasive species remains a priority for land stewards and managers at a leading international conservation organization. Contrary to recent accusations, land stewards at The Nature Conservancy distinguish between nonnative and nonnative invasive species and dedicate a substantial proportion of their conservation resources to management of the latter. Managers feel justified in their management choices because of the ecological and aesthetic impacts of nonnative, invasive species and would increase management if resources allowed. For whatever reason – skepticism, mixed messages, inertia – TNC operations on the ground are not yet substantially modified. We found that anti-invasion activities continue apace, and resource expenditures have even increased in some instances. Particular campaigns are featured in TNC’s Magazine and website (e.g., Reimers 2014, Smith 2014). Perhaps it is too early for management changes to be manifested in response to NCS and the New World Order, or perhaps its mandates for activities on the ground are not yet clear. Or possibly the critics are unconvincing and the paradigm is not actually shifting.

Acknowledgments

We thank the TNC staff for their time and responses to the survey, and two anonymous reviewers for their comments.

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Supplementary material 1

TNC Non-native Species Management Survey

Authors: Sara. E. Kuebbing, Daniel Simberloff

Data type: Adobe PDF file

Explanation note: Online survey to assess land managers nonnative species management strategies.

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Supplementary material 2

Survey Responses by Question

Authors: Sara. E. Kuebbing, Daniel Simberloff

Data type: Adobe PDF file

Explanation note: Survey responses for all questions.

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