

Support for major hypotheses in invasion biology is uneven and declining

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Abstract

Several major hypotheses have been proposed to explain and predict biological invasions, but the general applicability of these hypotheses is largely unknown, as most of them have not been evaluated using a standard approach across taxonomic groups and habitats. We offer such an evaluation for six selected leading hypotheses. Our global literature review reveals that those hypotheses that consider interactions of exotic invaders with their new environment (invasional meltdown, novel weapons, enemy release) are better supported by empirical evidence than other hypotheses (biotic resistance, island susceptibility, tens rule). We also show that empirical support for the six hypotheses has declined over time, and that support differs among taxonomic groups and habitats. Our results have implications for basic and applied research, policy making, and invasive species management, as their effectiveness depends on sound hypotheses.

Keywords

Biological invasions, biotic resistance hypothesis, decline effect, enemy release hypothesis, invasional meltdown hypothesis, island susceptibility hypothesis, novel weapons hypothesis, tens rule

Introduction

Invasion biologists ... need to take a good hard look at the fundamental tenets of the discipline and ensure that our understanding is built on hard evidence rather than assumptions, or on theories that have equivocal empirical support.

Moles et al. 2012

Invasive species are those that have been introduced to regions beyond their native range, established in the wild, and spread substantially from their point of introduction (Lockwood et al. 2007; Blackburn et al. 2009; Hulme et al. 2009). They can threaten global biodiversity, introduce diseases, cause other ecological problems, or incur economic costs (Pimentel et al. 2005; Kettunen et al. 2009; Pyšek and Richardson 2010). Studying biological invasions promises to both help mitigate impacts by invaders and better understand basic principles of ecology and evolution (Sax et al. 2007). Although research on biological invasions has a long tradition, publications on this topic have been numerous only since the 1990s (Darwin 1859; Elton 1958; Cadotte 2006; Davis 2006; Richardson and Pyšek 2008). From this perspective, invasion biology is still a young discipline (Pyšek and Hulme 2009). Its major hypotheses are logical and appealing, but the extent of empirical evidence supporting them is largely unknown (Moles et al. 2012). Some overviews of the level of support for individual hypotheses exist (e.g. Jeschke and Strayer 2005; Liu and Stiling 2006; Levine et al. 2004; Moles et al. 2012), but to our knowledge, no comparative evaluation of multiple major hypotheses across the entire range of taxonomic groups of invading species and invaded habitats is currently available. Such an evaluation is a critical step for any discipline, as it identifies research gaps, discriminates hypotheses with substantial support from those lacking it, and defines future priorities.

In this article, we evaluate six major hypotheses in invasion biology. Our evaluation is based on a standardized literature analysis across animals and plants in terrestrial and aquatic habitats. The six focal hypotheses represent a broad cross-section of the field and capture a variety of mechanisms thought to play a role in biological invasions:

1) *Biotic resistance hypothesis*: ecosystems with high biodiversity are more resistant against invaders than ecosystems with low biodiversity (Elton 1958; Levine and D'Antonio 1999; Lonsdale 1999; Mack et al. 2000; Maron and Vilà 2001; Shea and Chesson 2002; Levine et al. 2004; Fridley et al. 2007; Jeschke and Genovesi 2011). This formulation of the biotic resistance hypothesis is also known as diversity-invasibility hypothesis. It is important not only for invasion biology but also for the question of whether diversity affects ecosystem stability (Ives and Carpenter 2007).

2) *Island susceptibility hypothesis*: invasive species are more likely to become established and have major ecological impacts on islands than on continents (Elton 1958; Simberloff 1995; Shea and Chesson 2002; Jeschke 2008). This hypothesis is related to

the biotic resistance hypothesis, as continents typically have higher biodiversity than islands, mainly due to geographic and thus evolutionary isolation.

3) *Invasional meltdown hypothesis*: the presence of invasive species in an ecosystem facilitates invasion by additional species, increasing their likelihood of survival or ecological impact (Simberloff and Von Holle 1999).

4) *Novel weapons hypothesis*: in the exotic habitat, invasive species can have a competitive advantage against native species because they possess a novel weapon, i.e. a trait that is new to the resident community of native species and therefore affects them negatively (Callaway and Aschehoug 2000; Callaway and Ridenour 2004). Prime examples of novel weapons are plant biochemicals with allelopathic effects, but traits of other organisms can be considered as novel weapons as well, e.g. toxic substances produced by invasive cane toads (*Bufo marinus*).

5) *Enemy release hypothesis*: the absence of enemies in the exotic range is a cause of invasion success (Maron and Vilà 2001; Keane and Crawley 2002; Mitchell and Power 2003; Torchin et al. 2003; Colautti et al. 2004; Liu and Stiling 2006; Blumenthal et al. 2009).

6) *Tens rule*: approximately 10% of species successfully take consecutive steps of the invasion process: about 10% of species transported beyond their native range will be released or escape in the wild (they are called introduced species or casuals); about 10% of these introduced species will be able to establish themselves in the wild; and about 10% of species established will become invasive/pest species (Williamson and Brown 1986; Williamson 1996; Jeschke and Strayer 2005; Jeschke 2008).

There are other major hypotheses in invasion biology, but these six represent a broad cross-section of the discipline (Shea and Chesson 2002; Hierro et al. 2005; Dietz and Edwards 2006; Richardson and Pyšek 2006; Lockwood et al. 2007; Pyšek et al. 2008; Blackburn et al. 2009; Catford et al. 2009; Atwood and Meyerson 2011; Moles et al. 2012). Two of the hypotheses focus on ecosystems into which invaders were introduced (biotic resistance and island susceptibility), one focuses on the invaders themselves (tens rule), and the remaining three focus on invader-ecosystem interactions (invasional meltdown, novel weapons, enemy release).

Methods

Using the Web of Science, we systematically searched the literature for empirical studies across taxonomic groups and habitats that tested each of the six hypotheses. Our search terms consisted of: (1) a specific search term for each hypothesis, and (2) a general search term to restrict results to studies on non-native species. The following

general search term was applied for all hypotheses: (alien OR exotic OR introduced OR invasive OR naturalized OR nonindigenous OR non-native). The exact full search terms for the hypotheses are given in Table 1. Literature searches were carried out between February and May 2010 (see Table 1) and returned more than 3500 articles. We screened the titles and abstracts of these articles to identify potentially relevant articles for our study. We then consulted the full texts of these articles and checked references cited therein to find further relevant articles that were not returned by the Web of Science search. All relevant empirical studies that we found with our systematic search were included in the analysis. Theoretical studies, reviews, and meta-analyses were not included. We did include studies cited in reviews and meta-analyses, however. Some articles are included more than once in our analysis, as they have tested several hypotheses or sub-hypotheses (see the hierarchy-of-hypotheses approach below). Our analysis is restricted to publications in scientific journals. We excluded book chapters because they are not currently included in the Web of Science. Book chapters are also not as easily accessible as journal papers. In total, we identified 371 empirical tests of the focal hypotheses.

The hierarchy-of-hypotheses (HoH) approach

We used a novel approach to analyze these 371 empirical tests. This approach, which we call hierarchy-of-hypotheses (HoH) approach, captures the complexity of major hypotheses by hierarchically structuring them into smaller sub-hypotheses. We developed this approach for invasion biology, but it can be easily applied to other disciplines

Table 1. Search terms for hypotheses.

Hypothesis	Search term	Date of search
Biotic resistance, island susceptibility [†]	(biotic resistance OR resistance hypothesis OR diversity-invasibility hypothesis OR island susceptibility) AND <i>general search term</i> [‡]	19 Feb 2010
Invasional meltdown	meltdown AND <i>general search term</i>	18 Mar 2010
Novel weapons	(“novel weapon*” OR “allelopathic advantage against resident species” OR AARS) OR ((weapon* OR allelopath*) AND <i>general search term</i>)	26 May 2010
Enemy release	enemy release AND <i>general search term</i>	17 Feb 2010
Tens rule	(tens rule OR establishment success) AND <i>general search term</i>	19 Feb 2010

[†] The search for these related hypotheses was combined.

[‡] The general search term was: (alien OR exotic OR introduced OR invasive OR naturalized OR nonindigenous OR non-native).

as well. Researchers empirically testing a major hypothesis do not usually test it in all of its complexity. Most major hypotheses are not even empirically testable as such, as they are too broad and unspecific. As a result, researchers typically test a certain sub-hypothesis of the major hypothesis, although they are rarely explicit about this restriction. The HoH approach reflects this observation by formulating hypotheses in a way that makes them better testable (see the formulations of our six focal hypotheses), and by further dividing them into testable sub-hypotheses, as far as necessary. For example, the formulation “ecosystems with a high biodiversity are more resistant against invaders than ecosystems with a low biodiversity” which we used for the biotic resistance hypothesis is only one of the possible versions of this hypothesis. It can be further divided into sub-hypotheses, especially because biodiversity and resistance can be defined and measured in different ways. For example, a sub-hypothesis of the biotic resistance hypothesis is that native species richness (a measure of native biodiversity) is negatively correlated with establishment success of introduced species, defined as the fraction of introduced species that become established (an inverse measure of resistance). Further sub-hypotheses are formulated accordingly and included in the hierarchy of hypotheses (Fig. 1).

The HoH approach ensures application of the same yardstick to all empirical studies that are being evaluated. As different versions of a given hypothesis exist, it sometimes happens that two authors with similar empirical results come to opposite conclusions: the first author applies version *a* of a given hypothesis and finds that the

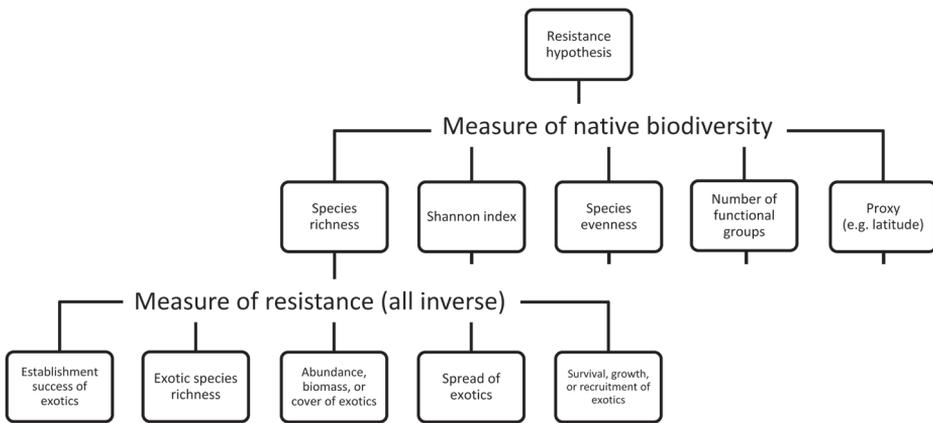


Figure 1. Example of a hierarchy of hypotheses. The biotic resistance hypothesis (as defined in the main body text) can be divided into sub-hypotheses according to the different measures (and thus definitions) of native biodiversity and resistance against invaders. Note that all measures of resistance typically applied are inverse measurements: the quantity measured reflects the susceptibility of ecosystems against invaders, so its inverse reflects resistance against invaders. Each of the five sub-hypotheses corresponding to different measures of biodiversity branch into five sub-sub-hypotheses corresponding to different measures of resistance against invaders. For illustrative purposes, the second branching is only shown for one sub-hypothesis (species richness as a measure of biodiversity).

data are in line with this version, whereas the second author applies version *b* of the hypothesis and finds the data are not in line with this version. Such discrepancies lead to a bias if all studies' conclusions are simply adopted, as is typically done in ordinary vote counting. The HoH approach solves this problem by comparing each study's results to standard criteria.

Similarly, the HoH approach ensures that only studies are included that test the major hypothesis as formulated and represented in the HoH. For example, the biotic resistance hypothesis is sometimes formulated in a broader way than we did here, by also considering effects of disturbance on resistance against invaders (e.g. Mack et al. 2000; Jeschke and Genovesi 2011; and references therein). We did not include studies investigating such effects, even though many of these studies include the term “resistance hypothesis” in their title or abstract. We do not claim that the formulation of the resistance hypothesis proposed here is the correct one. We do not even think there is a single correct formulation of a given hypothesis. It is, however, important to clearly state the hypothesis one is addressing, and the HoH approach helps doing that.

The HoH approach can be combined with fully quantitative analyses, e.g. meta-analyses based on effect sizes (Arnqvist and Wooster 1995; Gurevitch and Hedges 2001). Effect sizes can be extracted from published empirical tests of individual sub-hypotheses and be combined to evaluate support for a given major hypothesis. However, when comparatively evaluating multiple major hypotheses, one is often confronted with very heterogeneous data that can sometimes not all be reduced to a single effect-size metric, such as *d* (Gurevitch and Hedges 2001) or a correlation coefficient. A comparative evaluation would only be meaningful if a single effect-size metric can be used for all hypotheses. This was not the case for our hypotheses, hence we applied a semi-quantitative analysis, counting the number of studies that either support, question/oppose, or are undecided/inconclusive about each sub-hypothesis.

Let us look at an example to illustrate how studies were classified as supporting, questioning, or being undecided. For the sub-hypothesis of the biotic resistance hypothesis that is depicted in the lower left of Fig. 1, a study showing a significant (or otherwise statistically supported, e.g. by means of information-theoretic or Bayesian approaches) negative relationship between establishment success of exotics and native species richness would support this sub-hypothesis; a study showing a positive relationship or not detecting any relationship (with sufficient sample size to detect one) between establishment success of exotics and native species richness would question the sub-hypothesis; and a study showing inconsistent relationships, e.g. for different experimental setups, would be undecided about this sub-hypothesis. A study showing a relatively clear negative relationship, but without statistical support (e.g. due to low sample size), would also be classified as undecided; however, such studies are rare. For each study that we analyzed, we noted whether its conclusion regarding a given sub-hypothesis is based on statistical analysis that went beyond simple descriptive statistics such as means, medians, or standard errors. Typical examples of such statistical analysis are null-hypothesis significance testing or information-theoretic or Bayesian approaches. Taking aside empirical tests of the

tens rule, the conclusions of 293 out of 297 tests (98.7%) were based on statistical analysis defined in this way.

For the tens rule, the situation was a bit different, as many authors did not statistically compare their observed transition probability to those predicted by the tens rule. It is not commonly agreed upon if the data should be used to calculate a confidence interval for the transition probability and then see if it overlaps with the 10% value predicted by the tens rule, or if alternatively the observed transition probability should be compared to the 5–20% range suggested by Williamson (1996). These approaches are similar (as the 5–20% range was derived by considering confidence intervals and other aspects), but they are not the same. We followed Williamson's suggestion; hence, if a study reported a transition probability between 5 and 20%, we classified it as supporting the rule; if the transition probability was <5% or between 20.01 and 25%, we classified the study as undecided; and if the transition probability was >25%, we classified the study as questioning the tens rule.

In following the HoH approach, we subdivided the different hypotheses and sub-hypotheses as far as necessary, i.e. hypotheses and sub-hypotheses that were tested only by a few studies were not further divided (Table 2). In principle, our approach allows evaluation of empirical support for each sub-hypothesis. Such a detailed analysis of each sub-hypothesis is beyond the scope of this article, however, where we want to focus on overall support for the six major hypotheses. We thus summed up figures of empirical support for each sub-hypothesis to give total numbers for each major hypothesis. We then contrasted the hypotheses to assess whether they were empirically supported in general, whether differences in support exist among taxonomic groups (plants, invertebrates, vertebrates) or habitats (terrestrial, freshwater, marine), and whether published tests are lacking for specific taxonomic groups or habitats.

Table 2. All hypotheses and sub-hypotheses evaluated in this study.

Hypothesis no.	Description [†]
1	Biotic resistance hypothesis ($n = 129$)
1.1	Sub-hypothesis with species richness as measure of native biodiversity ($n = 114$)
1.1.1	Sub-sub-hypothesis with establishment success of exotics as inverse measure of resistance ($n = 14$)
1.1.2	Sub-sub-hypothesis with exotic species richness as inverse measure of resistance ($n = 57$)
1.1.3	Sub-sub-hypothesis with abundance, biomass, or cover of exotics as inverse measure of resistance ($n = 30$)
1.1.4	Sub-sub-hypothesis with spread of exotics as inverse measure of resistance ($n = 3$)
1.1.5	Sub-sub-hypothesis with survival, growth, or recruitment of exotics as inverse measure of resistance ($n = 10$)

Hypothesis no.	Description [†]
1.2	Sub-hypothesis with Shannon index as measure of native biodiversity ($n = 1$)
1.3	Sub-hypothesis with species evenness as measure of native biodiversity ($n = 2$)
1.4	Sub-hypothesis with number of functional groups as measure of native biodiversity ($n = 8$)
1.5	Sub-hypothesis with a proxy (e.g. latitude) as measure of native biodiversity ($n = 4$)
2	Island susceptibility hypothesis ($n = 9$)
3	Invasional meltdown hypothesis ($n = 30$)
4	Novel weapons hypothesis ($n = 23$)
5	Enemy release hypothesis ($n = 106$)
5.1	Sub-hypothesis comparing invaded and native range of exotic species [‡] ($n = 30$)
5.1.1	Sub-sub-hypothesis saying that exotics are less infested in invaded than native range ($n = 21$)
5.1.2	Sub-sub-hypothesis saying that exotics show a lower degree of damage in invaded than native range ($n = 9$)
5.2	Sub-hypothesis comparing exotic species with native species [‡] ($n = 45$)
5.2.1	Sub-sub-hypothesis saying that exotics are less infested than native species ($n = 23$)
5.2.2	Sub-sub-hypothesis saying that exotics show a lower degree of damage than native species ($n = 22$)
5.3	Sub-hypothesis comparing invasive exotic species with non-invasive exotic species [‡] ($n = 9$)
5.3.1	Sub-sub-hypothesis saying that invasive exotics are less infected than non-invasive exotics ($n = 3$)
5.3.2	Sub-sub-hypothesis saying that invasive exotics show a lower degree of damage than non-invasive exotics ($n = 6$)
5.4	Sub-hypothesis saying that exotic species profit (e.g. in terms of biomass) from the absence of enemies ($n = 22$)
6	Tens rule ($n = 74$)
6.1	Sub-hypothesis on the following transition in the invasion process: transport → release (or casual) ($n = 7$)
6.2	Sub-hypothesis on the following transition in the invasion process: release (or casual or introduction) → establishment ($n = 50$)
6.3	Sub-hypothesis on the following transition in the invasion process: establishment → spread (or pest) ($n = 17$)

[†] Only those sub-hypotheses are described that were actually tested by the identified studies. Hypotheses and sub-hypotheses that were only tested by a few studies were not further subdivided for our analysis.

[‡] See van Kleunen et al. (2010) for these types of comparisons.

The decline effect

We also investigated the presence of a 'decline effect', asking if empirical support for each hypothesis has declined over time. The term 'decline effect' describes the phenomenon that published empirical support for a given hypothesis declines over time. The strength of empirical support is often measured as effect size, so in such cases a decline effect is observed if published effect sizes decline over time. The phenomenon itself has been known since the 1930s but is receiving wide attention now (Lehrer 2010; Schooler 2011). It still lacks an official name (Lehrer 2010) but many researchers call it 'decline effect' (Schooler 2011). One reason for the recent interest in this phenomenon is due to better availability of long-term data. The effect is known from several disciplines, especially medicine where the decline in apparent effects of different pharmaceuticals is alarming (Lehrer 2010). A decline effect has also been reported in psychology (Lehrer 2010; Schooler 2011) and ecology and evolution (Poulin 2000; Jennions and Møller 2002; Lehrer 2010), but to our knowledge it has never been investigated in the context of biological invasions.

Several possible explanations for the decline effect have been discussed; one is publication bias, as supporting evidence for a new hypothesis is more interesting and can thus be published easier and faster than data that question a new hypothesis (Poulin 2000; Jennions and Møller 2002; Lehrer 2010). Only when a hypothesis has become established (because it has been supported by several published studies) does it become interesting to publish data that question the hypothesis. Such changing motivations to publish supporting and questioning evidence for a given hypothesis can lead to a publication bias that changes over time, which can in turn lead to a decline effect.

A decline effect can also be caused by a bias in study organisms or systems (Poulin 2000; Jennions and Møller 2002). It seems likely that a certain phenomenon is first noticed and described for an organism (or system) where it is particularly strong, as researchers are especially interested in a phenomenon if it is of high importance for their model organism. After publishing their findings, they will look for the phenomenon in related organisms where its presence appears likely as well. As a result, early studies on a certain hypothesis tend to be done for organisms where positive results are expected. Only later will other organisms be tested.

There are also statistical and psychological explanations for the decline effect (Jennions and Møller 2002; Lehrer 2010). Most decline effects probably have multiple reasons, but differentiating them has been hampered by data availability (Schooler 2011). In fact, decline effects are currently unknown in most disciplines and await better investigation.

We tested for a decline effect by comparing the level of empirical support for early vs. recent studies of each major hypothesis. 'Early' studies are the first ~50% of studies published on a given hypothesis, whereas 'recent' studies are the latest ~50% of studies. The cut-off point was determined to be as close as possible to 50%; it was not exactly 50%, as papers published in the same year were not split. We then performed a Generalized Linear Model (GLM) analysis for ordinal dependent variables (multino-

mial distribution, cumlogit link; PASW Statistics 2010, version 18.0.2) with ‘support’ (questioned, undecided, or supported) as dependent variable and with ‘hypothesis’ (biotic resistance hypothesis, island susceptibility hypothesis, ...) and ‘time’ (early or recent studies) as predictor variables.

Results

Three of the six hypotheses have low support ...

We found that three of the six focal hypotheses are supported by >50% of available empirical tests (invasional meltdown: 77%; novel weapons: 74%; enemy release: 54%), whereas the other three hypotheses are supported by <30% of available empirical tests (biotic resistance: 29%; tens rule: 28%; island susceptibility: 11%; Fig. 2). Hypotheses with higher empirical support are those that consider invader-ecosystem interactions, whereas the other hypotheses do not include these interactions and focus on either invaders (tens rule) or ecosystems (biotic resistance, island susceptibility). Our literature search returned many more empirical tests of biotic resistance ($n = 129$), enemy release ($n = 106$), and the tens rule ($n = 74$) than of invasional meltdown ($n = 30$), novel weapons ($n = 23$), and island susceptibility ($n = 9$).

... and support is declining over time across hypotheses

Comparing early to recent empirical tests of the six hypotheses showed that empirical support for these hypotheses has declined over time (Fig. 3). According to a Generalized Linear Model (GLM), observed empirical support significantly differs (1) among hypotheses ($p < 0.001$, 5 DF, likelihood ratio chi-square = 60.52), thus statistically confirming differences mentioned in the previous section, and (2) between early and recent studies ($p < 0.05$, 1 DF, likelihood ratio chi-square = 4.84). In other words, the decline in empirical support shown in Fig. 3 is statistically significant across the six hypotheses. The decline’s intensity seems to differ among hypotheses – the differences in percent studies supporting each hypothesis between early and recent studies are as follows (depicted in Fig. 3): 5% for biotic resistance, 25% for island susceptibility, 41% for invasional meltdown, 25% for novel weapons, 10% for enemy release, and 10% for the tens rule. However, sample sizes are currently low for some hypotheses, so future tests are needed to clarify whether the decline’s intensity really differs among hypotheses.

Differences also exist among taxonomic groups and habitats

Empirical support for the tens rule is stronger for plants and invertebrates than for vertebrates (Fig. 4), whereas support for biotic resistance, enemy release, and invasional

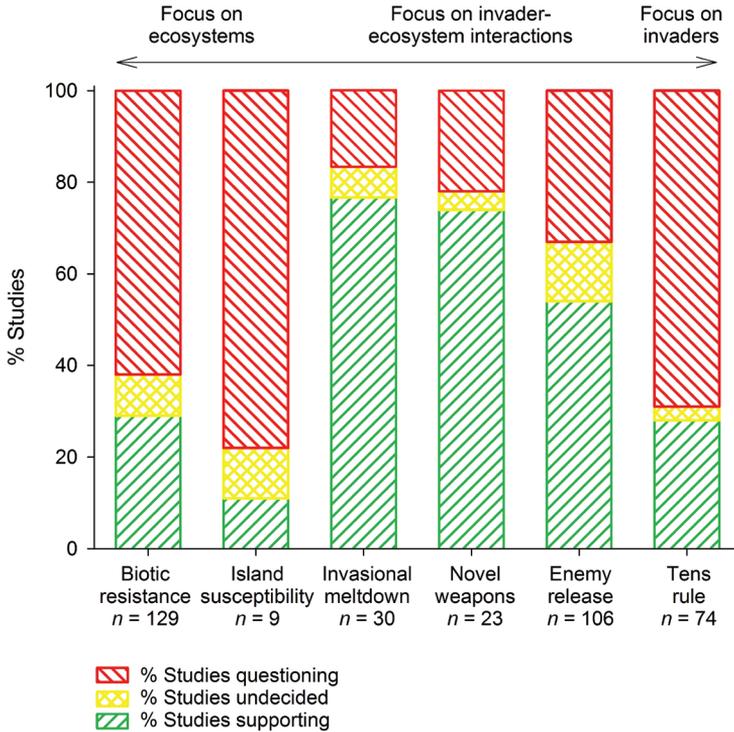


Figure 2. Overall level of empirical support for six of invasion biology’s major hypotheses. Hypotheses focusing on ecosystems where invaders were introduced are on the left (biotic resistance, island susceptibility), the tens rule which focuses on the invaders themselves is on the right, and hypotheses considering invader-ecosystem interactions are in between (invasional meltdown, novel weapons, enemy release).

meltdown does not significantly differ among taxonomic groups. For island susceptibility and invasional meltdown, a sufficient number of empirical tests are currently available only for one taxonomic group each (Fig. 4).

Comparing studies of invaded terrestrial, freshwater, and marine habitats shows significant differences for biotic resistance and the tens rule: for these two hypotheses, support from marine studies is strongest (Fig. 5). We found no significant differences for invasional meltdown and enemy release, and did not test habitat differences for the two remaining hypotheses (island susceptibility and novel weapons), as empirical tests of these hypotheses are currently available only for terrestrial habitats.

The big picture

In sum, invasional meltdown currently has the highest level of support of the six hypotheses, with consistently high levels of support across taxa and habitats. Support for this hypothesis has substantially declined over time, but numbers of empirical studies

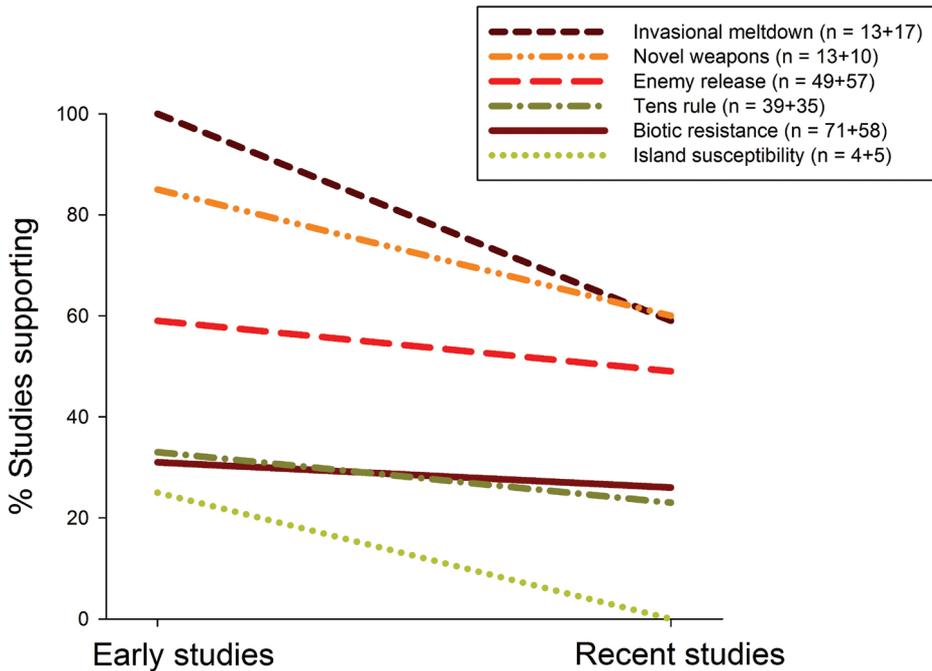


Figure 3. Level of empirical support for six of invasion biology’s major hypotheses, subdivided for early and recent studies. Numbers of early and recent studies for each hypothesis are provided in the legend. To improve the figure’s clarity, only % studies supporting each hypothesis are shown here, and other studies are not further divided into those questioning and being undecided about each hypothesis (as in Figs 2, 4, 5). When statistically testing for a decline effect, however, all three levels of empirical support (supported, undecided, questioned) were considered. The decline effect shown here is statistically significant (Generalized Linear Model, likelihood ratio test, $p < 0.05$).

are still limited, so it would be premature to assume that the decline will continue to be so substantial.

The novel weapons hypothesis reaches a similar overall level of support as the invasional meltdown hypothesis, but it has been tested only for terrestrial plants, so its applicability to animals and aquatic habitats is unclear. Empirical support for this hypothesis has markedly declined over time as well.

Enemy release has received mixed support from existing tests, and again, most tests have focused on terrestrial plants. When comparing early and recent studies on enemy release, however, there is only a slight decline in the frequency of supporting studies.

Biotic resistance, island susceptibility, and the tens rule all have low levels of empirical support. Support for biotic resistance is low across taxonomic groups, but most marine studies have reported supporting evidence: 55% of marine empirical tests have supported the biotic resistance hypothesis. The tens rule performs poorly overall, but

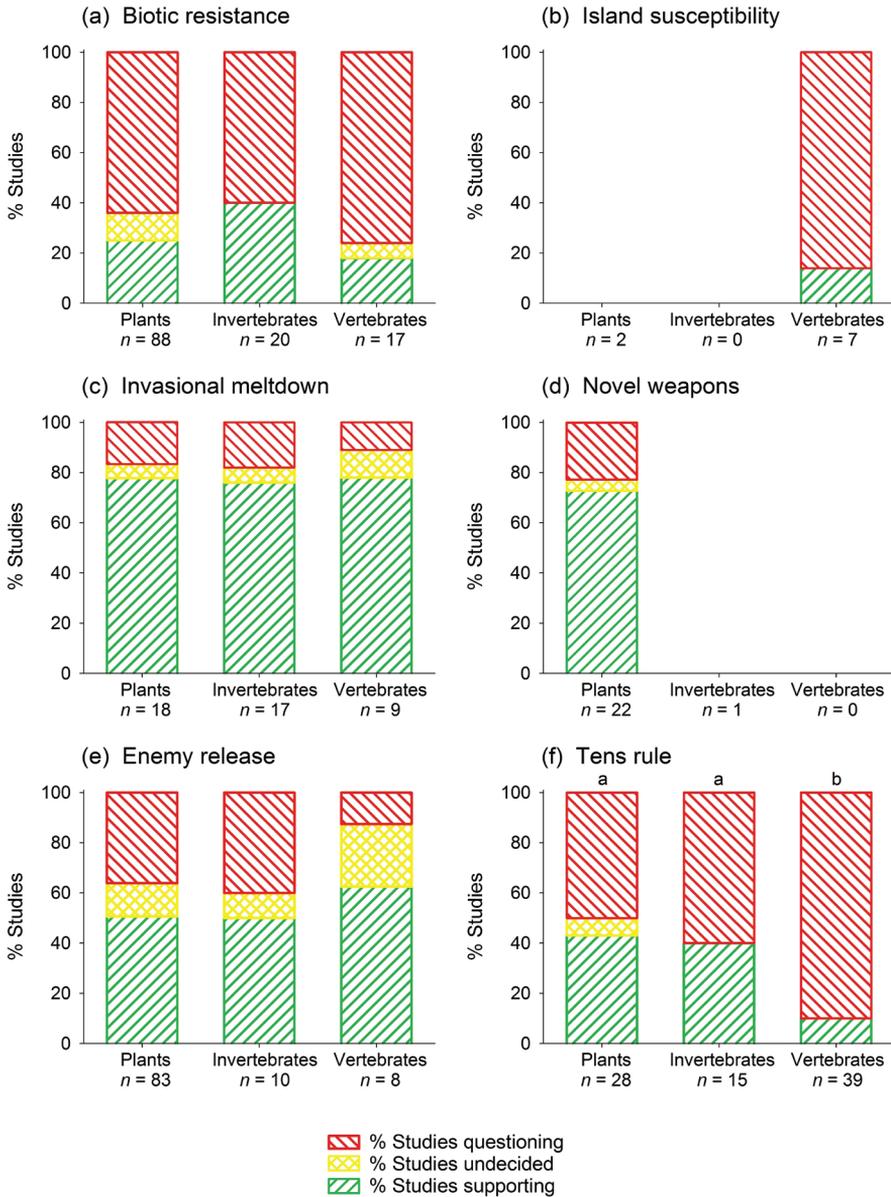


Figure 4. Level of empirical support for six of invasion biology's major hypotheses, subdivided by taxonomic groups. The few cross-taxonomic studies that covered more than one of the three indicated taxonomic groups were counted for each group. Missing bars indicate lacking data: bars are shown only if at least five studies were carried out for a given hypothesis and taxonomic group. Letters in (f) indicate significant differences between taxonomic groups (U tests, $p < 0.01$). No significant differences were observed for the other hypotheses.

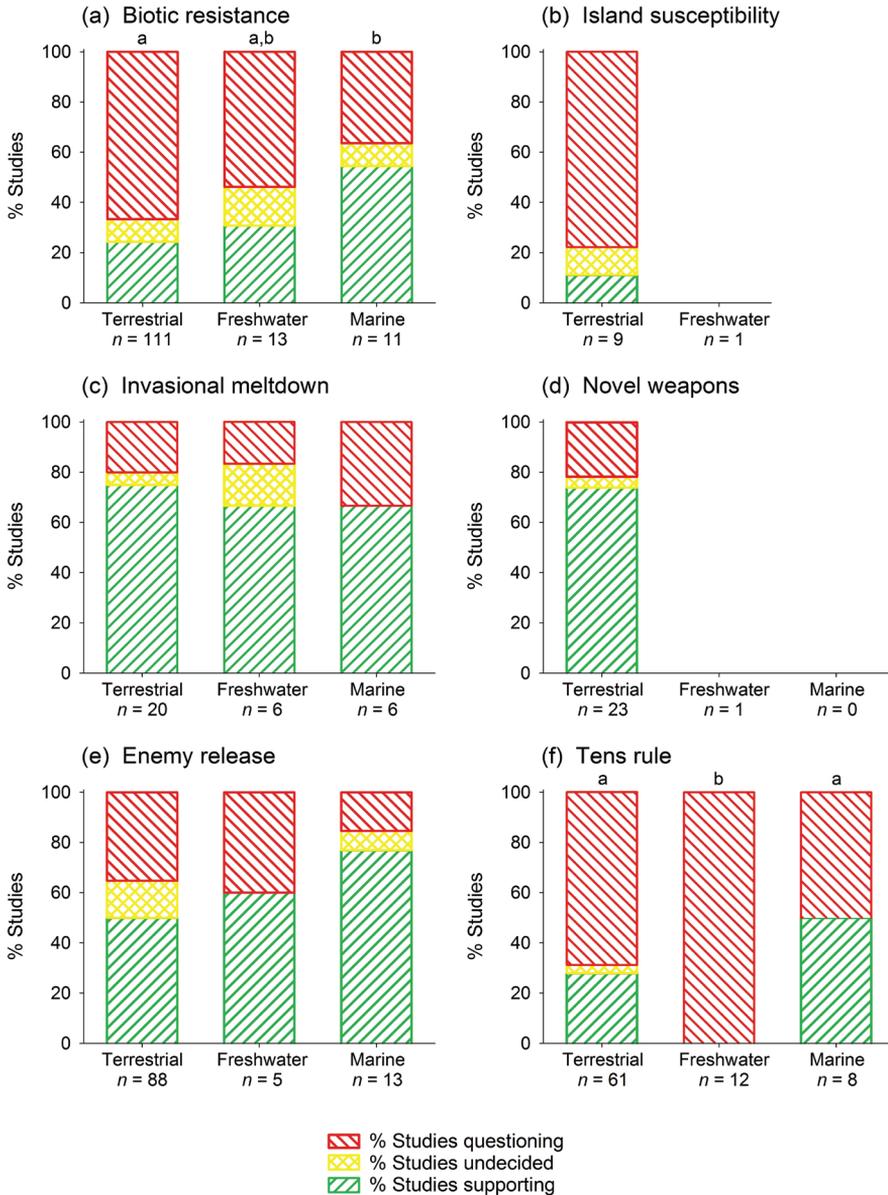


Figure 5. Level of empirical support for six of invasion biology's major hypotheses, subdivided by habitats. The few studies that covered more than one of the three habitats were counted for each habitat. Missing bars indicate lacking data: bars are only shown if at least 5 studies were carried out for a given hypothesis and habitat. Note that the island susceptibility hypothesis is not applicable to the marine habitat. Letters in (a) and (f) indicate significant differences between habitats (U tests, $p < 0.05$). No significant differences were observed for the other hypotheses.

significant differences among taxa and habitats exist. The decline in empirical support for biotic resistance and the tens rule is small. The island susceptibility hypothesis has been tested mainly for terrestrial vertebrates (78% of all studies, 67% on birds), and studies indicate almost no support, especially recent studies. This hypothesis has not been sufficiently tested for plants, invertebrates, and freshwater habitats.

Discussion

Our results suggest that empirical support for some major hypotheses in invasion biology is both doubtful and has declined over time. This is unfortunate, as both effective policy making and management of invasive species rely on sound hypotheses. For instance, many policy and management decisions depend on the potential risk posed by species introductions, which partly depends on the probability that introduced species become invasive. This probability is predicted to be low by the tens rule, but actual data suggest it can be high, depending on the taxonomic group and habitat in question. A similar consideration applies to the biotic resistance hypothesis which posits that diverse ecosystems are relatively resistant against invaders. Based on this hypothesis, it could be argued that no specific policy or management actions are required to protect diverse ecosystems, as they are inherently 'safe'. Yet, currently available evidence questions the resistance hypothesis, hence diverse ecosystems need to be protected against harmful invaders, too.

To our knowledge, this is the first broad comparative evaluation of multiple major invasion hypotheses. Several previous studies evaluated individual hypotheses, e.g. empirical data from plants were found to only weakly support the disturbance hypothesis which was not evaluated here (Moles et al. 2012), and empirical data from animals were found to not support the tens rule (Jeschke and Strayer 2005; Jeschke 2008). Here, we further show that empirical data from plants more frequently support the tens rule than data from animals, yet levels of support for this hypothesis do not exceed 50% across taxonomic groups and habitats. The tens rule differs from our other focal hypotheses in that it only attempts to describe a pattern rather than trying to explain it. Nonetheless, it is not better supported by empirical tests than the other hypotheses. Meta-analyses exist on the biotic resistance and enemy release hypotheses, but to our knowledge only for exotic plants (Liu and Stiling 2006; Levine et al. 2004). Using a broader perspective, our analysis provides insights into the relative success of hypotheses across taxonomic groups and habitats, suggesting which hypotheses are better supported by empirical evidence than others. It is possible to compare our results to previous analyses and reviews for each of our focal hypotheses, but going into details for each specific hypothesis is beyond the scope of this article. Instead, our review aims to provide results that help to broadly evaluate the current state of the discipline and suggest priorities for future research.

Four solutions to current challenges in invasion biology

What is the way forward? Our results suggest four possible solutions to current challenges in invasion biology:

Solution 1 – Fill existing gaps in empirical tests of hypotheses. It is crucial to fill existing gaps in empirical studies on invasion biology's major hypotheses, such as those identified in this study for specific taxonomic groups and habitats (Figs 4, 5).

Solution 2 – Specify hypotheses for taxa and habitats. Despite the current shortage of empirical tests, our results already show that hypotheses differ in their applicability among taxonomic groups and habitats. It might be too much to expect that most hypotheses apply across all taxonomic groups and habitats. On the other hand, if a hypothesis applies only to a single taxon consisting of a few species (e.g. a genus or family), it is not too useful for the field in general. What would be useful instead is a general hypothesis that can be specified for given taxa and habitats, so that these variants of the hypothesis provide reliable predictions for each taxon and habitat. The hierarchy-of-hypotheses approach together with a subdivision of empirical tests according to taxonomic groups and habitats, as done here, are first steps into this direction. They allow us to better understand which hypothesis (and which version of which hypothesis) works best for which taxonomic group and habitat.

Solution 3 – Consider invader-ecosystem interactions. Hypotheses that do not consider invader-ecosystem interactions (e.g. biotic resistance, island susceptibility, tens rule) might benefit if they are revised to consider such interactions. For example, the biotic resistance hypothesis could be revised by considering key-lock effects (e.g. facilitation) between resident and introduced species, or the tens rule could be revised by considering the composition and functional structure of resident communities. Our results suggest that hypotheses addressing invader-ecosystem interactions have more potential to improve our understanding of biological invasions than those focusing solely on either invaders or characteristics of the new environment. The idea to develop a balanced approach to biological invasions that addresses both invaders and the new environment has been formulated before (Roy 1990; Heger and Trepl 2003; Richardson and Pyšek 2006; Schaefer et al. 2011), but to our knowledge, the current study is the first to bolster this idea with evidence across taxonomic groups and habitats. Further studies are, of course, needed to test if the predictive power of hypotheses really improves if they are revised to include invader-ecosystem interactions.

Solution 4 – Reject revised hypotheses if they do not work. Some hypotheses will not be rescued by a specification for taxonomic groups and habitats (solution 2), a consideration of invader-ecosystem interactions (solution 3), or another form of revision. Hypotheses that still lack support after revision should be discarded, as we should not

waste our time and resources to continue testing hypotheses that simply do not work. Instead, we should use our creativity to come up with fresh ideas and new hypotheses.

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A review of the influence of root-associating fungi and root exudates on the success of invasive plants

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Abstract

Plant-fungal interactions are essential for understanding the distribution and abundance of plants species. Recently, arbuscular mycorrhizal fungal (AMF) partners of non-indigenous invasive plants have been hypothesized to be a critical factor influencing the invasion processes. AMF are known to improve nutrient and moisture uptake, as well as disrupt parasitic and pathogenic microbes in the host plant. Such benefits may enable invaders to establish significant and persistent populations in environments previously dominated by natives. Coupling these findings with studies on invader pathogen-disrupting root exudates is not well documented in the literature describing plant invasion strategies. The interaction effects of altered AMF associations and the impact of invader root exudates would be more relevant than understanding the AMF dynamics or the phytochemistry of successful invaders in isolation, particularly given that AMF and root exudates can have a similar role in pathogen control but function quite differently. One means to achieve this goal is to assess these strategies concurrently by characterizing both the general (mostly pathogens or commensals) and AM-specific fungal colonization patterns found in field collected root samples of successful invaders, native plants growing within dense patches of invaders, and native plants growing separately from invaders. In this review I examine the emerging evidence of the ways in which AMF-plant interactions and the production of defensive root exudates provide pathways to invasive plant establishment and expansion, and conclude that interaction studies must be pursued to achieve a more comprehensive understanding of successful plant invasion.

Keywords

Arbuscular mycorrhizal fungi, plant invasions, root exudates

Introduction

Approximately 10% of non-indigenous plant species can significantly suppress or eliminate native populations (henceforth ‘invaders’), and have successfully established in regions around the globe, while others merely become naturalized and integrate with the native community (Richardson et al. 2000a). Explaining why some species are widely successful, with large impacts on native biota, is an active area of research (Rejmánek and Richardson 1996, Mack et al. 2000, Inderjit et al. 2008). In particular, herbaceous species such as *Vincetoxicum rossicum*, *Euphorbia esula*, *Cirsium arvense*, *Alliaria petiolata*, *Polygonum cuspidatum*, and *Phragmites australis* (dog-strangling vine, leafy spurge, Canada thistle, garlic mustard, Japanese knotweed, and common reed, respectively) have established widespread monocultures in Canada and become ecologically and economically problematic, threatening both native biota and reducing agricultural production (Lorenz 1991, Meekins and McCarthy 1999, Ailstock et al. 2001, Klironomos 2002, Kruger-Mangold et al. 2002, Cappuccino 2004).

The hypotheses explaining invader success are multifaceted and continue to evolve (Mack et al. 2000, Inderjit 2005). Explanations of why certain invaders successfully establish in novel ranges generally include reduced negative interactions or key facilitative interactions that allow certain species to overcome biotic barriers to invasion. A number of these theories attempt to explain invader success as a function of fungal associations, both beneficial and detrimental, or as a function of invader root exudates, which have also been shown to cause significant shifts in the life-history strategy of native plants such as reproductive timing and characteristics (Parepa et al. 2012). The Enemy Release hypothesis proposes that invaders flourish because they are released from natural enemies that are either not found or have not successfully adapted to the invader in the novel environments, including fungal pathogens (Keane and Crawley 2002). The Novel Weapons hypothesis suggests that invaders possess chemical exudates that are harmful to previously unexposed native organisms in the novel environment, thus disrupting plant communities and abetting invader establishment (Callaway et al. 2008). The Diversity-stability hypothesis holds that more diverse ecosystems are likely to contain at least one or more species prone to thriving under conditions of environmental perturbation, and are therefore able to fill niches of competitors that falter under such conditions—thus increasing resistance to invasion (Tilman and Downing 1994). Consequently, dampening of diversity due to the success of invading species can also result in ‘invasional meltdown’, whereby the proliferation of one invader can increase the likelihood of successful establishment of subsequent invaders (Simberloff and Von Holle 1999). As well, the concept of a ‘unifying theory’ was put forward by Hallett (2006), suggesting that successful invasions result from a combination of dislocation from antagonistic relationships, the availability of generalist microbial mutualists in the novel environment, and allelopathy.

The benefits attributed to mutualisms such as mycorrhizal (root-fungal) associations have recently become well represented in the literature (Johnson et al.

1997, Bever 1999, Smith and Read 2008). Similarly the advantages derived from powerful allelopathic and anti-pathogenic root exudates (novel weapons) have been linked to successful plant invasions. These effects are generally categorized as either i) benefits derived from associations with arbuscular mycorrhizal fungi (AMF) which are symbionts in the order Glomales, known to enhance nutrient and moisture uptake as well as provide protection from pathogens, or ii) benefits derived from root exudates that contain antibiotic (allelopathic, antipathogenic or anti-feedant) or signaling compounds. These investigations have typically been carried out in isolation of one another, although it is evident that there are distinct interdependencies and interactions operating between mutualistic and chemical strategies (Newsham et al. 1994, Klironomos 2002, Hallett 2006). Invaders are also suspected of being fungal generalists, a trait that can result in successful establishment and significant seedling recruitment, leading to the formation of monocultures and loss of community diversity.

My objective in this review is to summarize the current understanding of plant-fungal associations in conjunction with root phytochemistry dynamics of successful invaders, and to suggest where research needs to be expanded in order to demonstrate the potential interdependencies between these two areas of research (see Figure 1). Knowledge of how they jointly influence invaders will further our understanding of processes supporting highly successful plant invasions.

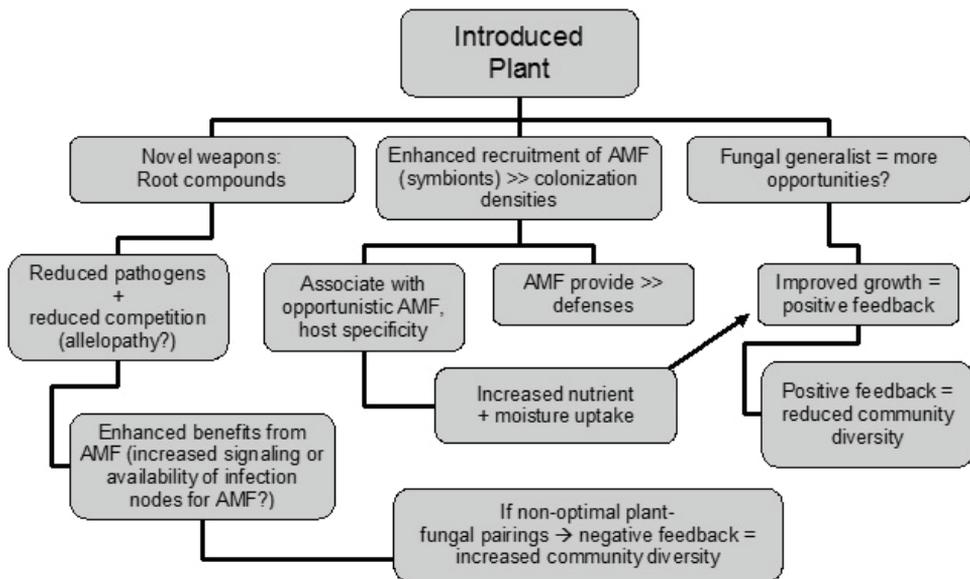


Figure 1. Possible dynamics within the rhizosphere and local soil environments subsequent to invasion by introduced plant species. The invader may possess novel or unique root compounds, an enhanced ability to establish associations with AMF, and/or the ability to associate with a wide range of beneficial fungi (some opportunistic P providers) relative to native plants.

AMF and invaders

Research related to plant-fungal association influences on invaders tends to bifurcate into two groups: investigations on fungi providing beneficial mutualisms, such as AMF and ectomycorrhizal fungi, and investigations on the impacts of parasitic/pathogenic fungi. In the case of the former, hyphal networks (vegetative filamentous structures) of mycorrhizae can extend as much as 200 times farther into the soil than the roots they colonize (Quinn, 2011), providing increased resource availability that the plant would not otherwise have access to. Fungal partners may also mediate plant-to-plant transfer of carbohydrates and other nutrients among different individuals of the same plant species, as well as among different plant species within the community by linking together the roots of two or more plants. Such mycorrhizal communities are referred to as “common mycorrhizal networks” (Selosse et al 2006, Egerton-Warburton et al 2007). These generalist networks can result in the creation of inoculum reservoirs that promote selective seedling establishment within a plant community. However, plants differ in their responses to mycorrhizal fungi, and the fungi differ in their ability to distribute nutrients between coexisting plants. Arbuscular mycorrhizal plant-fungal symbioses form highly specialized nutrient-exchange structures (vesicles and arbuscules) at the plant-fungus interface and are recognized as being a particularly beneficial group of root endophytes. Plants associating with AMF are typically more competitive and better able to tolerate environmental stresses than are non-mycorrhizal or poorly colonized plants (Biermann and Linderman 1983, Daniell et al. 2001, Bianciotto and Bonfante 2002, Vandenkoornhuysen et al. 2002, Bonfante 2003, Brundrett 2004, DeBellis and Widden 2006, Greipsson and DiTommaso 2006, Parniske 2008, Shah et al. 2010), and AMF associations may contribute to the diversity of plant communities.

Bever (2002) noted that there is evidence of negative feedback on abundance of a given plant species resulting from the asymmetries between plant and AM fungus: plant-fungal associations are not always established in such a way as to maximize the benefit realized by either partner. For example, in the case where Plant-1 grows best when partnered with AMF-1, AMF-1 may be more inclined to establish associations with Plant-2, and so on. This dynamic is thought to contribute to the successful coexistence of a variety of plant species functioning within species guilds, thus preventing dominance by one or a few species. In this scenario, negative feedback due to non-ideal plant-fungal pairings is a mechanism that promotes diversity in plant communities. An introduced plant may disrupt this type of system balance where it differentially promotes or disrupts fungi in the plant community, suggesting that AMF research must be coupled with investigations on established plant-fungal community pairings within a local environment. These as well as other findings would indicate that species identity is also relevant to invasion biology when considering plant-fungal mutualisms (Crawley et al. 1999).

Root structure is another factor for consideration with regard to plant-AMF associations. Seifert et al. (2009) noted that introduced plants generally had finer root architecture relative to their native European counterparts, a characteristic consistent

with a tendency to correspondingly increase reproductive biomass in newly introduced species. These findings suggest a reduced colonization by AMF in introduced plant variants, as finer roots are more typical of species with lower mycorrhizal responsiveness (Baylis 1975, Hetrick et al. 1992, Vogelsang and Bever 2009). Given that the structure and development of mycorrhizal hyphae is substantially altered in the presence of roots of host plants (Brundrett 2009), a shift in plant species representation post-invasion has the ability to significantly impact local AMF.

Plant community structure can be influenced by the presence/absence, density, and species richness of AMF in local soils, as plants can differ in their response to AMF colonization (Klironomos 2003). Less competitive plant species may establish a foothold within a community where they otherwise may have failed to persist in the absence of AMF in the rhizosphere, suggesting that AMF promote plant coexistence and therefore contribute to community diversity (Moora and Zobel 1996, van der Heijden et al. 1998). For example, when small heal-all *Prunella vulgaris* plants were inoculated with AM fungi, they were able to successfully compete with larger neighbouring woodland strawberry *Fragaria vesca* plants that they had failed to successfully compete with pre-inoculation in greenhouse experiments (Moora and Zobel 1996). AMF associations would therefore increase biodiversity by decreasing the interspecific suppression of small plants by larger neighbours. It should be noted that fine-scale studies look at the impact of composition and diversity of AMF rather than just presence or absence of AM fungi (Hart et al. 2003), and therefore these studies consider factors such as host specificity and have more relevance for later-successional stages of invader range expansion. It has been proposed that AMF may be either disrupted or harnessed by aggressive invasive plant species, resulting in altered native fungal communities in the soil as the invader attains dominance in the system (van der Heijden et al. 1998, Daniell et al. 2001, Griepsson and DiTommaso 2006, Bastias et al. 2007, Curlevski et al. 2010).

The mechanisms by which AMF association provides advantages to successful invaders as they establish and attain dominance in a novel range typically include: i) increased general AMF colonization density of invaders relative to natives can lead to improved nutrient and moisture uptake and increased competitiveness, ii) host specific AMF that support plant community structure and diversity can be significantly altered by invaders, and iii) pathogen protection by AMF may be provided selectively to invaders.

Key findings for each category are summarized below.

AMF colonization, dependence, and density

Some aggressive plant invaders have been observed to sustain significantly increased AMF colonization densities relative to local native plants in the field. An example of this is *Vincetoxicum rossicum*, colloquially known as dog-strangling vine (DSV) because its twining tendencies lead to dense interconnected mats of impenetrable vegetation.

DSV has been shown to be more densely colonized by mycorrhizal fungi than co-occurring native plants in Henderson Harbour, New York (Greipsson and DiTommaso 2006, Smith et al 2008). Smith et al. (2008) used bright field microscopy to detect evidence of significantly greater fungal colonization of DSV relative to leek *Allium ampeloprasum* bait plants (frequently used as a predictor of AMF density in soils), and local natives such as milkweed *Asclepias syriaca*, Canada goldenrod *Solidago canadensis*, or naturalized blueweed *Echium vulgare* L. Additionally, DSV colonized by fungal partners had a significantly greater total biomass relative to DSV grown in sterilized soil reamended with AMF-free microbial wash (Smith et al. 2008).

Soil microbial communities have been observed to change progressively as invasion advances from year to year (Wolfe and Klironomos 2005, Batten et al. 2006), and it has been suggested that invaders benefit disproportionately from symbiotic mutualists, particularly in disturbed sites (Reinhart and Callaway 2006, Sun and He 2010). Plants are capable of controlling the density of mycorrhiza representation by root growth, digestion of old interface hyphae in plant cells, or altered root system formation (Brunnett 2008). These types of mechanisms may be at play in the field when invasions occur, followed by shifts in fungal colonization patterns. A study by Liang et al. (2004) showed a significant positive correlation between invasion time and AMF colonization rate of goldenrod *Solidago canadensis*, a successful invader in China, though there was differential colonization evidenced by the different AMF species, depending on the environmental characteristics. They noted that one species of AMF, *Glomus mosseae*, demonstrated increased colonization density over time in *S. canadensis*, while another AMF species, *G. constrictum*, showed decreased colonization density over time. It was suggested that *G. constrictum* may be an early successional species, and *G. mosseae* a late-successional species. The authors concluded that AMF aided establishment and proliferation of *S. canadensis* in its introduced range in China.

In contrast to the above studies, soil/AMF conditioning studies and bioassays by Vogelsang and Bever (2009) demonstrated that the invasive herbaceous forb Italian thistle *Carduus pycnocephalus* exhibited strong growth in soil lacking arbuscular mycorrhizal fungi, as well as in soil conditioned by a diverse mix of non-native plant species. As well, *C. pycnocephalus* growth was inhibited by the soil that best promoted the native herbaceous forb California cudweed *Gnaphalium californicum*. Mycorrhizal density investigations showed a reduction of AMF in the invader-conditioned soil relative to the native-conditioned soil, suggesting that in some cases invader species do not promote the growth of mycorrhizal fungi in the same way that native species do. Given that most crop plants are hosts to AMF, this association is potentially an important resource for agriculture (Sieverding 1991, Oehl et al. 2003), and should be considered for further study.

AMF host specificity, richness, and opportunism

Given the observed low AM fungi:host plant ratio (i.e. approx. 150 described AMF species to 300,000 plant species), it is generally assumed that there is a high functional

redundancy among AM fungal species, and therefore low host specificity (Klironomos 2000). However, it has been shown that there are differential phosphorus uptake and pathogen protection responses that are highly plant- and fungus-species specific for different plant-fungus combinations (van der Heijden et al. 1998, Helgason et al. 2002, Duponnois et al. 2005, Gustafson and Casper 2006, Gogoi and Singh 2011). Optimal plant-AM fungi combinations are more likely to be established where higher AMF species diversity is present. As such, mycorrhizal fungal species richness has been shown to affect plant productivity, and observed shifts and loss of diversity in AMF groups post invasion could impact plant community structure (Maherali and Klironomos 2007). It has been observed that certain aggressive invading plant species can alter the existing native fungal communities in a densely invaded environment, thus disrupting native plant communities (Helgason et al. 2002, Kourtev et al. 2002, Mummey et al. 2005, Batten et al. 2006, Greipsson and DiTommaso 2006, Hawkes et al. 2006, Stinson et al. 2006, Meinhardt and Gehring 2012). Hawkes et al. (2006) described a dramatic shift in the composition of AMF communities in native North American grasses *Nassella pulchra* and *Lupinus bicolor* subsequent to invasion by introduced grasses *Avena barbata* and *Bromus hordeaceus*. There was a shift away from species originally colonizing natives toward those colonizing the invader, and fungal species richness in the natives was reduced as a result of the shift (Hawkes et al. 2006). Compositional changes in AM fungi pre- versus post-invasion supports host specificity as a factor, given that reduction in native plant species representation correspondingly reduced the associating fungi. The authors concluded that invading plants could influence the network of mycorrhizal fungi available to natives, thus providing a mechanism for successful establishment and subsequent invasion. Similarly, Mummey et al. (2005) used terminal restriction fragment length polymorphism (T-RFLP) and multivariate analyses to show that AMF communities associating with *Dactylis glomerata*, a common forage grass species naturalized in mid-western US, shifted to reflect the community composition associated with *Centaurea maculosa*, a noxious weed native to eastern Europe, post invasion. As well, Stinson et al. (2006) observed that the non-mycorrhizal exotic invasive forb garlic mustard *Alliaria petiolata* suppressed both AMF and ectomycorrhizal fungi, the latter being instrumental in tree seedling establishment and growth. Disruption of AMF by successful invaders could result in loss of natives dependent on such species, supporting competitive exclusion by the invader.

Vandenkoornhuyse et al. (2003) used PCA analyses to determine plant-fungal association patterns generated by T-RFLP using AMF-specific primers. They demonstrated that three different co-occurring grass species (*Agrostis capillaries*, *Festuca rubra*, and *Poa pratensis*) were differentially colonized by AMF phylotypes, suggesting that recognition mechanisms exist that confer fungal-host plant specificity. Another study by Helgason et al. (2002) similarly investigated host specificity in mycorrhizal fungal associations using small-subunit rRNA gene amplification and sequencing. They found that the only species of AMF colonizing field collected *Acer pseudoplatanus* in North Yorkshire (UK) was *Glomus hoi*, while several other species of AMF (*Scutellospora dipurpureus*, *Archaeospora trappei*, and *Glomus sp.* isolate UY1225) were found

differentially colonizing four neighbouring plant species (*Rubus fruticosus* agg. L., *Epilobium angustifolium* L., *Ajuga reptans* L. and *Glechoma hederacea* L.). The *Glomus hoi* consistently outperformed other AM fungi in improving P uptake in all the woodland plants in greenhouse experiments, thus enhancing the growth of these mycorrhizal plant species (Helgason et al. 2002), particularly in P-limited environments. Consequently, disruption or promotion of specific AMF by invaders would significantly impact existing plant community structure.

Greipsson and DiTommaso (2006) predicted that highly invasive species such as *Vincetoxicum rossicum* will tend to associate with fast-growing opportunistic or highly beneficial AMF species, enabling rapid establishment of invaders in their novel range. *Glomus* species have been shown to comprise a subset of such AMF because they have the advantage of readily forming anastomoses (connections between hyphae) with other fungi and are able to colonize via hyphal fragments, whereas other AM fungi typically require intact mycelia to colonize host plants, or direct infection from spore dispersal (Figure 2) (Biermann and Linderman 1983, Giovannetti et al. 1999, Fumanal et al. 2006). *Glomus intraradices*, *G. caledonium*, and *G. mosseae* were observed to form anastomoses between hyphae originating from the same spore as well as different spores from the same isolate, while this was not the case for *Gigaspora* or *Scutellospora* spp. tested (also AMF) (Giovannetti et al. 1999). As such, these *Glomus* species have been shown to behave opportunistically relative to commonly co-occurring AMF (Giovannetti et al. 1999, Helgason et al. 2002). As well, preferential P uptake by *G. intraradices* has been observed in several studies (Graham and Eissenstat 1994, Appoloni et al. 2008, Van Aarle et al. 2009), suggesting that this species of AMF would be particularly beneficial in low P environments. DSV was observed to associate with *G. intraradices* and *G. caledonium* in field studies in Southern Ontario, where several co-occurring native plants were not observed to form such associations based on molecular investigation of root-colonizing AMF communities (Bongard et al., unpublished data).

Glomus species with demonstrated ability to colonize roots via hyphal fragments also appeared to be more beneficial in promoting plant growth over *Gigaspora* species (Bever et al. 2009), which have no demonstrated ability to colonize roots via hyphal fragments (Biermann and Linderman 1983, Bever et al. 2009). Maherali and Klironomos (2007) also noted that members of the Glomaceae (includes *Glomus* species) have demonstrated reduced pathogen abundance in roots; however, Gigasporaceae generally enhanced nutrient uptake in their plant hosts. Highly beneficial influences on plant growth was observed in the shrub *Acacia holosericea* inoculated with *Glomus intraradices* in a study by Duponnois et al. (2005), who observed that *G. intraradices* optimized P solubilization and uptake from mineral phosphate. Maldonado-Mendoza et al. (2001) observed that a phosphate transporter gene (GiPT) in the extra-radical mycelium of *G. intraradices* was expressed during associations with carrot *Medicago truncatula* roots. Their analyses revealed that GiPT expression is regulated in response to phosphate concentrations in the environment and modulated by the overall phosphate status of the mycorrhiza, suggesting that *G. intraradices* can detect phosphate

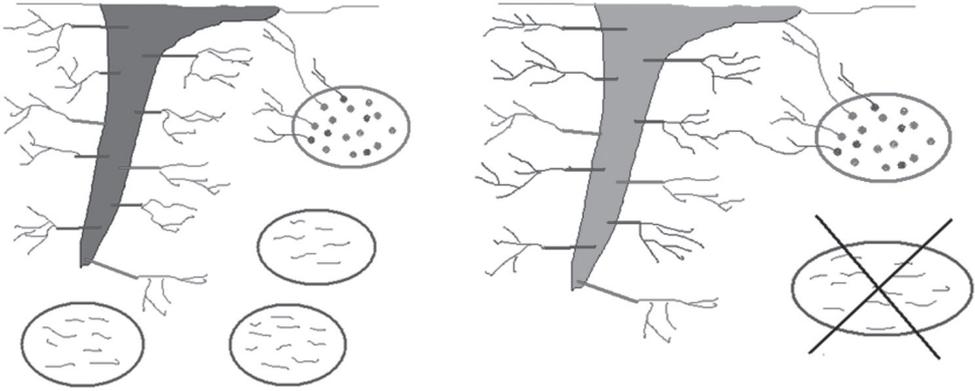


Figure 2. The root on the left represents an invasive plant that is able to form associations with opportunistic AM fungal fragments, while the root on the right cannot form such associations, but must form associations with hyphae of germinating spores or intact mycelia (i.e. established hyphal networks). The ability to form these opportunistic associations with hyphal fragments would be highly advantageous in mechanically disrupted sites.

levels in the external environment, as well as having an internal phosphate sensing mechanism. This ability in the propagative units among glomalean families would provide enhanced early successional establishment opportunities for plants capable of forming associations with these species (Giovannetti et al. 1999). In heavily mechanically disrupted sites, mycorrhizal networks may be in a slow state of recovery and therefore respond in a manner similar to early successional behavior (Hart et al. 2003), suggesting that colonization via mycelial fragments, or opportunistic *Glomus spp.*, may be the predominant method of forming associations. Where invaders are preferentially colonized by this subset of AMF, establishment advantages would be gained.

While plant-fungal host specificity has been described in various studies, it has been observed that some invaders are most likely fungal generalists (Richardson et al. 2000b, Miller 2008, Moora et al. 2011), a characteristic that may provide advantages for establishment and spread in a novel environment. For example, DSV has been observed to form associations with a greater variety of fungal colonizers relative to proximal native plants, and has also demonstrated greater fungal colonization densities (Smith et al. 2008, Bongard et al., unpublished data).

AMF pathogen protection

AMF species with the ability to protect their host plants from a variety of microbial pathogens have been described in the literature (Newsham et al. 1994, Borowicz 2001, Klironomos 2002, Pozo and Azcón-Aguilar 2007, van der Putten et al. 2007, Appoloni et al. 2008, Mogg et al. 2008, Krüger et al. 2009, Wehner et al. 2009). It has been suggested that plants associating with these highly beneficial AMF could

have defensive enzyme and chemical production induced in their roots by their mycorrhizal partners, and subsequent damage to roots of non-host plants may occur while host roots remain protected (Brundrett 2004, Bais et al. 2006). Disruption of protective AMF by invaders would increase the susceptibility of natives that typically benefit from this type of protection, while the invader may simultaneously benefit from enemy release provided in the novel environment (Keane and Crawley 2002). Possible differential exploitation of these AM fungi by invaders would also provide advantages in the novel environment.

In a study using meta-analysis of AMF and plant pathogen data gathered from papers published between 1970 and 1998, it was determined that AMF tended to decrease the harmful effects of fungal pathogens (Borowicz 2001), and were shown to reduce parasitic nematode *Meloidogyne incognita* damage in white clover *Trifolium repens* L. (Habte et al. 1999). In experiments testing three AMF *Glomus* species (*G. aggregatum*, *G. mosseae*, and *G. intraradices*) for their relative effectiveness in providing protection against nematode infestation, *G. intraradices* was found to provide the most effective protection (Habte et al. 1999). The authors noted that colonization by *G. intraradices* in particular was enhanced in the presence of nematodes, and that nematodes did not have a significant influence on plants colonized by *G. intraradices*. *G. intraradices* has been described as a promiscuously associating generalist (Graham and Eissenstat 1994, Appoloni et al. 2008, Van Aarle et al. 2009).

Maherali and Klironomos (2007) found that increased Glomaceae richness in roots generally increased competition against pathogenic soil fungi in lab experiments. Shah et al. (2010) observed reduced Glomaceae representation in soils post invasion by two highly invasive European herbaceous species *Anthemis cotula* and *Conyza canadensis* (Asteraceae) using rhizosphere spore analysis, thus potentially resulting in the loss of AMF-induced pathogen protection to natives. As well, Vigo et al. (2000) observed that tomato plants *Lycopersicon esculentum* colonized by *Glomus mosseae* had reduced infection by the soil pathogen *Phytophthora parasitica* relative to plants not colonized by *G. mosseae*, with results showing 39% fewer infection loci from the pathogen seven days after zoospore inoculation with *G. mosseae*. These findings suggest that one mechanism by which AMF protect against fungal pathogen infection is via efficient colonization of the plant infection points or loci, which are limited in number. As such, this hypothesis supports the necessity of combining AMF colonization density studies with those investigating AMF host specificity and AMF as agents of protection. Wright and Upadhyaya (1998) hypothesized that glomalin, a glycoprotein unique to AMF, is used for pathogen protection of the AMF hyphae that extend into the soil and facilitate nutrient transfer. Sub-groups of AMF may also be activating defense mechanisms within the host plant: ‘defensin’, a cysteine-rich plant protein demonstrating anti-fungal activity was only upregulated in roots colonized by *G. intraradices* relative to roots that were colonized by *G. mosseae* (Wehner et al. 2009).

Though AMF have been shown to confer protection against pathogenic fungi, it has been observed that there could be synergistic and/or additive effects produced by different AMF species combinations (Gustafson and Casper 2006, Wehner et al. 2009).

Wehner et al. (2009) hypothesized that protection against pathogens could be due to the combined effects of increased access to nutrients provided by AMF, enhancement of plant defense mechanisms stimulated by AMF associations, and altered root architecture (increased lignin concentrations) as a response to AMF colonization. However, depending on the soil environment and other factors, AMF can function on a continuum from parasitism to facultative exploitation by their plant hosts (Brundrett 2004); hence environment nutrient load must also be considered. Because of the variability in the benefits provided by AM fungi, it is important to understand more about the dynamics of the local fungal community as a whole, not just AM fungal density or dominant species type.

In planning studies on invasion success, it would be useful to consider multiple effects attributable to simultaneous colonization by AMF as well as other fungi, plant traits such as root architecture, and variation in benefits derived from different AMF species, which may or may not afford unique chemical protection to colonized roots (Seifert et al. 2009). Having a more complete picture of the plant-fungal dynamics would offer a better view to plant invasion processes, as well as elucidate how AM fungal specialists interact with the general (non-AMF exclusive) fungal community in supporting plant diversity and competition.

Fungal pathogens and invader root exudates

Fungal pathogens

Some invasive plant species have demonstrated significantly reduced infection by fungal pathogens in their introduced range relative to their native range, an effect that may not be directly attributed to associations with AMF (Keane and Crawley 2002, Callaway et al. 2003, Mitchell and Power 2003). This could be explained by the Enemy Release hypothesis described previously. However, Eppinga et al. (2006) used mathematical modeling to account for distribution patterns and success of the invasive species *Ammophila arenaria* (marram grass or European beach grass) in California that resulted from *A. arenaria*'s ability to tolerate accumulation of local soil pathogens rather than enemy release. The authors hypothesized that while the accumulation of pathogens may initially limit invader abundance in the novel environment, it might feed back more negatively to the native plant community. As such, they concluded that in California *A. arenaria* accumulated local generalist pathogens that had a more negative effect on the native plant species than on *A. arenaria*. Alternatively, Blumenthal et al. (2009) examined the effects of fungal pathogens hosted by invaders in the US relative to the effects of the same plants in their native European ranges. Their results suggested that plants from resource-rich native environments (strong competitors) are more likely to experience release from pathogens in their non-native range than plants originating in low resource habitats (stress-tolerant plants). The authors hypothesized that successful invaders will possess high growth characteristics and demonstrate a lower pathogen burdens in their introduced range.

Root exudates

Plant root exudates are substances that alter the conditions of the rhizosphere by changing pH levels and mineral availability via desorption, as well as influencing the growth and interactions of microorganisms (Rovira 1969). Plant-plant interactions are often mediated by root exudates (Bais et al. 2006, Parepa et al. 2012). Allelopathy, the suppression of germination or growth of neighboring plants by the release of toxic secondary chemical compounds, has been explored as a pathway for successful plant invasion (Callaway and Aschehoug 2000, Inderjit 2005, Callaway and Howard 2007, Antunes et al. 2008, Douglass 2008, de Souza et al. 2010, Cantor et al. 2011). De Souza et al. (2010) used multiscale mathematical models to determine that plant invasion patterns and success based on allelopathy depends on both the nature of the local native plants in the invaded site, as well as the nature of the invader phytotoxins (root exudates). Both the celerity and success of invasion increase in the presence of weakly resistant and relatively homogeneous native plants and effective invader phytotoxins. For example, in the case of spotted knapweed *Centaurea maculosa* in the western US, invasion is particularly successful due to the phytotoxin (-)-catechin exuded from roots, which inhibits germination and triggers the death of root systems of susceptible native grass species (Bais et al. 2003). Allelopathy has also been suggested as a means of successful expansion of invaders in North America (Callaway et al. 1999, de Souza et al. 2010), including DSV (Milbrath 2010, Milbrath et al. 2011), as well as invaders in other countries such as Japanese knotweed *Fallopia japonica* in Wales (Hollingsworth and Bailey 2008) and Canada goldenrod *Solidago canadensis* in China (Zhang et al. 2009). Extracts of *S. canadensis* roots were also shown to significantly suppress the growth of soil oomycete and fungal pathogens *Pythium ultimum* and *Rhizoctonia solani* relative to extracts of common local native plants in culture experiments (Zhang et al. 2010).

Studies have shown that root exudates of invaders can decrease colonization by pathogenic fungi in their novel range (Bais et al. 2006, Mogg et al. 2008). However, the impacts of root pathogens can range from reduced plant growth and fecundity to total plant failure; the interaction of plant and pathogen depends upon the class of pathogen as well as the defense-relative-to-growth strategies of the plant. Strong competitors in resource rich environments tend to allocate resources to growth (large leaf area) rather than defense, but often cope relatively well with a high pathogen load, whereas stress-tolerant competitors that excel in resource poor environments tend to make use of defensive root exudates that keep pathogens in check (Seastedt 2009). Consequently, in resource rich novel environments with reduced plant-specific pathogens (release from enemies), invaders are able to become strong competitors, while in disturbed, resource poor novel environments, invaders can gain an advantage when their root compounds (anti-pathogenic or anti-feedant chemicals) are particularly effective against pathogens and herbivores.

Invader root exudates may disrupt AM fungal associations of nearby natives, thereby decreasing competitiveness (Vierheilig et al. 2003, Stinson et al. 2006), or even comprise chemicals that are both allelopathic toward neighbouring plants and disruptive

to AMF (Zhang et al 2010, Hale 2011). Zhang et al. (2007) demonstrated that root extracts of *S. canadensis* added to several native plants in China (*Echinochloa crusgalli*, *Kummerowia striata*, and *Ageratum conyzoides*) significantly inhibited AMF colonization by several *Glomus* species common to natives one year post invasion. Broeckling et al. (2008) observed that two model plant species, *Arabidopsis thaliana* (non-mycorrhizal Brassicaceae) and *Medicago truncatula* (AMF-associating), demonstrated the ability to maintain associations with local soil fungus communities consistently over time, while not being similarly capable of maintaining non-resident soil fungus associations. The authors compared the resident soil fungus phylotype community profiles between native and non-native plant conditioned soils (or soils conditioned with their root exudates) using real-time PCR. The presence of non-native plants growing in soils, or their root exudates alone, influenced the fungal community by both positively and negatively impacting the relative abundance of individual phylotypes, while the native plants (or their root exudates) maintained consistent fungal community profiles over multiple plant generations. Accordingly, where an invader brings fungi new to the introduced range, natives dependent on resident fungal communities that are prone to disruption would be disadvantaged. As such, root exudates may serve as a selective agent enabling regulation of the fungal community in the rhizosphere (Broeckling et al. 2008). Roots of common milkweed *Asclepias syriaca* (in the same family as DSV, Asclepiadaceae) gained significant biomass when grown in DSV-conditioned soil relative to plants grown in uninvaded soils (DiTommaso 2006). This was attributed to a possible escape from fungal pathogens induced by the phenthorindolizadine alkaloid chemical (–)-antofine exuded by DSV roots (Mogg et al. 2008). DSV has demonstrated antifungal activity against plant pathogenic yeast-like and filamentous fungi, as well as broad-host-range plant pathogens such as *Fusarium spp.* (Mogg et al. 2008). These findings suggest that there is a strong likelihood that fungal communities associating certain aggressive invaders will differ from those associating with native plants that do not demonstrate similar phyto-chemistry, resulting in native community disturbance.

Stinson et al. (2006) reported that the mutualistic associations between native tree seedlings and mycorrhizal fungi were disrupted by the non-mycorrhizal European native garlic mustard *Alliaria petiolata*, resulting in decreased seedling survival. They found that hardwood seedlings grown in garlic mustard-conditioned soil showed significantly reduced AMF colonization of roots and slower growth than those grown in non-garlic mustard conditioned soil, based on lab experiments conducted in Waterloo, Ontario. The authors attributed the ability of *A. petiolata* to dampen seedling growth of AMF-dependent competitors to the glucosinolate root exudates manufactured by these members of the Brassicaceae family. These findings were supported by Callaway et al. (2008), who found that the anti-fungal influence of *A. petiolata* had far greater inhibitory effects in North America than in their native European ranges. Root exudates that are relatively harmless to resistant mycorrhizal symbionts in the home range as a result of adaptation, may therefore be disruptive to native mutualists in the introduced range, and indirectly suppress the plants that rely on them (Callaway et al. 2004).

Some plant root exudates have also been shown to increase the signaling pathways attracting AM fungi, and consequently increase hyphal growth and colonization by these beneficial symbionts (Vierheilig 2004, Akiyama et al. 2005, Greipsson and DiTommaso 2006). A net increase in fungal biomass was observed in the experiments by Broeckling et al. (2008) when non-resident root exudates were added to resident plant treatments (*Arabidopsis thaliana* and *Medicago truncatula*), which the authors suggested could be attributed to increased production of signaling compounds in the roots triggered by the root exudates. Where invaders are selectively able to benefit from increased AMF colonization due to unique signaling compounds, they will experience advantages relative to proximal native plants, particularly in moisture- or P-limited environments.

Prevailing plant-fungal post invasion responses are listed in Table 1.

Table 1. Alternative plant-fungal strategies of invasive plants in a novel range. Shifts in soil fungi will vary in the plant rhizosphere as invasion progresses, depending on the nature of the fungi introduced by or associating with the invader, and the root exudates of the invader. Native versus invader responses are suggested.

Increase in AM fungal diversity and abundance	Decrease in AM fungal diversity and abundance	No change in fungal community	Main references
Invader has caused fungal shift or brought novel AMF, increasing AMF diversity/abundance, potentially resulting in increased nutrient uptake or pathogen protection. Natives may benefit if host specificity is either suitable or not a factor	Invader introduced a novel AMF species into the soil environment that is unable to colonize natives, but becomes pervasive Invader sustains decreased AMF colonization density or richness, while simultaneously increasing potential fungal pathogen load in rhizosphere	Invader is able to form associations with existing AMF but experiences escape from resident pathogens in novel environment Invader is a fungal generalist that associates with a variety of local AMF without causing significant disruption or change to fungal community	Appoloni et al. 2008, Bastias et al. 2007, Batten et al. 2006, Beckstead and Parker 2003, Callaway et al. 2003, Graham and Eissenstat 1994, Hawkes et al. 2006, Keene and Crawley 2002, Meinhardt and Gehring 2012, Mogg et al. 2008, Moora et al. 2011, Mummey and Rillig 2006, Richardson et al. 2000b, Van Aarle et al. 2009
Increased signaling compounds (root exudates) added to rhizosphere by Invader enables increased AM and general fungal colonization Benefits to natives vary	Invader releases toxins that decrease general and AM fungal persistence in the rhizosphere; allelopathy; possibility of decreased pathogen protection and/or nutrient/moisture uptake in proximal natives	Invaders capitalizes on alternate strategies to dominate the system such as allelopathy, ability to compete in resource-depleted or disturbed environments more successfully than natives	Antunes et al. 2008, Bais et al. 2003, Broeckling et al. 2008, Callaway et al. 2004, Callaway et al. 2008, Douglass 2008, Parniske 2008, Pozo and Azcón-Aguilar 2007, Stinson et al. 2006
Reduced fungal pathogens occupying root infection loci due to infusion of novel chemicals by Invader; AMF are able to take up residence in newly available loci of Invader (possibly natives)	Invader is able to form associations with existing AMF via hyphal fragments or anastomoses, where natives are not and such AMF species become pervasive in the rhizosphere	Increased fungal pathogens occupying infection loci cause reduced AMF colonization – net effect is decrease in symbionts but increase in commensalists and pathogens	Biermann and Linderman 1983, Callaway et al. 2003, Daniell et al. 2001, Giovannetti et al. 1999, Helgason et al. 2002, Mogg et al. 2008, Vigo et al. 2000

AMF and invader root exudate interaction studies

Although most authors have focused their research on either the benefits/anti-pathogenic properties of AMF or the benefits associated with invader plant root exudates independently, there are some examples of much needed interaction studies. Newsham et al. (1994) explored the relative effects ascribed to both AMF and fungal pathogens (potentially controlled by either AMF or root exudates) in the field, and determined how the two groups of fungi interacted to determine plant response and fitness. They found that while AMF colonization of winter annual grass *Vulpia ciliata* ssp. *ambigua* was reduced by the application of anti-fungicides, the root, shoot, total plant biomass, and phosphorus inflows were unaffected. The authors hypothesized that this somewhat unexpected finding arose due to the fungicidal depression of pathogenic root-inhibiting fungi such as *Fusarium oxysporum* or *Embellisia chlamydospora* isolated in the roots of *V. ciliata* that might otherwise be compromising plant growth and fecundity. Newsham et al. (1994) concluded by suggesting that the main benefit supplied by arbuscular mycorrhizal fungi to the plant was apparently protection from pathogenic attack, not phosphorus uptake. Significant reductions in fecundity resulted from the root pathogen associations in *V. ciliata*, though the infections were otherwise asymptomatic, presenting a specialized case of plant-pathogen interaction. The authors postulated that there was an interaction effect between the AMF and pathogenic fungi on some level, resulting in the ostensibly asymptomatic nature of the infection by pathogens. Observations of both sets of fungi in an invaded environment provide insights as to simultaneous shifts that could be instigated by exposure to invader root exudates or alterations to AMF initiated by the invader via diverse strategies.

In a study by Klironomos (2002), local fungal pathogens demonstrated a negative impact on growth and spread of rare plants when grown in local or home soil over time, while invasive plants demonstrated net positive growth when grown in their own local soil (and associated fungi), suggesting reduced susceptibility to local pathogens over time. The effects of AMF, however, did not differ between the rare and invasive plant groups. For both groups (native and invaders), AMF isolated from soil with a history of the same plant species had a more positive effect on plant growth than fungi isolated from a different plant host. With both groups of plants, inoculations using AM fungi from foreign plants rarely resulted in significant growth depressions. These findings suggest that most plants can potentially experience positive feedback with AMF communities, but this effect is not realized unless negative feedback resulting from pathogens is limited. This arises as a consequence of the differential effect of pathogenic fungi on invasive plants relative to rare plants, rather than a differential response to the AMF. When introduced to previously uncultured soil at low densities, plants that ultimately achieve high abundance do not seem to accumulate species-specific pathogens at the same rate as plants that remain in low abundance (Klironomos 2002). Pathogen accumulation in highly successful invasive plant species tends to happen more slowly than that of natives demonstrating low abundances due to negative feedback mecha-

nisms, including dense colonization by microbial pathogens (Bever 2002, Klironomos 2002, Mitchell and Power 2003, Inderjit and van der Putten 2010).

Studies investigating simultaneous shifts in both the general fungal community and AMF would provide clarification as to the role of each subsequent to plant invasion. In doing so, they would elucidate the combined mechanisms of invader root exudates invoking pathogen reduction via allelopathy, as well as the response of altered or introduced AMF subsequent to invasion. While other interaction study methods have been suggested, including use of microbial microarrays and detailed assays of root exudates to differentiate phytochemicals that signal AMF hyphenation and infection from those targeting disruption of rhizosphere pathogens (Bais et al. 2006), the use of molecular alternatives such as T-RFLP and other fingerprinting methods could provide the same result at a reduced effort. Primers targeting the general fungal community differentially from primers targeting AMF produce both a proxy for the rhizosphere response to allelopathic chemicals in invaders roots (shifts in general fungal community), as well as directly observable shifts in the AMF community.

Conclusions and future directions

It is necessary to design experiments that can account for the multiplicity of events taking place in local soil environments post invasion to gain a greater understanding of the fungal-chemical interactions in the rhizosphere (local scale), and within the ecosystem (regional scale) as the plant invasion progresses. Investigating fungal communities (both general and AMF) in pre- and post-invaded sites over time would be useful in determining how each factor, such as the state of AMF or non-AM fungi (comprised of fungal pathogens, commensalists, and mutualists), as well as the influence of invader root exudates or the interaction of all of these factors, might contribute to invasion success. For example, if amplified AMF colonization of invaders via increased signaling compounds were to confer a highly successful invasion pathway relative to the anti-pathogenic effects of invader root exudates, it would suggest that AMF inoculation may not be an effective restoration strategy. If the opposite were true, inoculation of a restored site with local AM fungi might be useful for the establishment of select re-introduced native plants, even where residual invader root exudates persist in the soil.

Investigations pairing a highly successful invader and a native plant species that are both known for producing allelopathic root exudates, along with non-allelopathic natives would be useful for AMF and general fungal phylotype comparisons using molecular analyses such as T-RFLP. The terminal restriction fragment analyses would enable comparison of the variation in both general and AM fungal communities, with the variable TRFs serving as a proxy for different fungal phylotypes associating with the plant groups. Where TRF data show a tendency for the general fungi associating with allelopathic plants to differ from the non-allelopathic plants in both natives and invaders, but not similarly for the AMF community, allelopathy is then recognized as a factor rather than the various scenarios linked to benefits associated

with increased or altered AMF post invasion. Where pathogenic fungi are being depressed or are well tolerated in invader plant roots but AM fungi are not similarly altered, it suggests that natives growing in an invaded patch may be susceptible to higher pathogen loads relative to natives that are unexposed, supporting the hypotheses of both Eppinga et al. (2006) and Klironomos (2002). Alternatively, natives may be more negatively affected by specific pathogen introductions associated with an invader than the invader itself. This effect will likely vary considerably for different native plant species, and individual investigations that are relevant to the invasion site would be informative. These types of molecular investigations also allow for comparisons of AMF diversity and density changes relative to those occurring in the general fungal community (including pathogens) as invasions progress. It would be expected that the general fungal community would be less diverse/abundant in invader roots relative to natives if pathogenic fungi are being depressed or virtually eliminated by phytochemicals (root exudates) in invaders that are not present in natives. As well, looking at the response of AM fungi relative to responses in the general fungal community would also potentially reveal the relative role of AMF as invasion progresses (Hawkes et al. 2006, Mummey and Rillig 2006).

In considering alteration of soil fungal communities by invasive plants, differential fungal association dynamics need to be quantified and compared, a process that is well supported by molecular investigation methods. Comparing both AMF and general fungal community fingerprints within a variety of native plant roots in pre- and post-invaded sites will provide insight as to whether shifts in AMF or the general fungal community (or both) are facilitating the invasion process. Molecular fingerprinting techniques could also be used to investigate variation in the diversity and density of fungal colonization during different stages of invader succession to determine which species are supporting the establishment and progression of invaders, particularly in light of the fact that delayed proliferation in time and space (due to Allee effect) has been observed in many invasive plant species.

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Does residence time affect responses of alien species richness to environmental and spatial processes?

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Abstract

One of the most robust emerging generalisations in invasion biology is that the probability of invasion increases with the time since introduction (residence time). We analysed the spatial distribution of alien vascular plant species in a region of north-eastern Italy to understand the influence of residence time on patterns of alien species richness. Neophytes were grouped according to three periods of arrival in the study region (1500–1800, 1800–1900, and > 1900). We applied multiple regression (spatial and non-spatial) with hierarchical partitioning to determine the influence of climate and human pressure on species richness within the groups. We also applied variation partitioning to evaluate the relative importance of environmental and spatial processes. Temperature mainly influenced groups with species having a longer residence time, while human pressure influenced the more recently introduced species, although its influence remained significant in all groups. Partial regression analyses showed that most of the variation explained by the models is attributable to spatially structured environmental variation, while environment and space had small independent effects. However, effects independent of environment decreased, and spatially independent effects increased, from older to the more recent neophytes. Our data illustrate that the distribution of alien species richness for species that arrived recently is related to propagule pressure, availability of novel niches created by human activity, and neutral-based (dispersal limitation) processes, while climate filtering plays a key role in the distribution of species that arrived earlier. This study highlights the importance of residence time, spatial structure, and environmental conditions in the patterns of alien species richness and for a better understanding of its geographical variation.

Keywords

Climate, dispersal limitation, energy, environmental filtering, human pressure, land-use, niche-based processes, propagule pressure

Introduction

Understanding the factors that determine the spatial distribution of exotic species is a primary objective of invasion ecology (Marini et al. 2009, de Albuquerque et al. 2011a, 2011b). Many factors have been postulated to influence invasiveness (the extent to which an introduced species is able to overcome various biotic and abiotic barriers, establish, proliferate, and disperse in a new environment) (Wilson et al. 2007).

Stochastic factors, including initial inoculum size, residence time, propagule pressure, and chance events (Richardson and Pyšek 2006), as well as, post introduction dispersal by human agency (Hodkinson and Thompson 1997, Kowarik 2003) are crucial for determining whether (or when) a species will invade. One of the most robust emerging generalisations in invasion biology is that the probability of invasion increases with the time since introduction ('residence time'). Residence time integrates a suite of factors (some directly affecting propagule pressure) that potentially affect the success of an alien species: the longer the species is present in the region, the higher the likelihood that more propagules are spread, and the probability of founding new populations increases (Rejmánek et al. 2005, Richardson and Pyšek 2006). The positive relationship between residence time and current distribution of alien species is evident in several studies (Rejmánek 2000, Castro et al. 2005, Hamilton et al. 2005, Pyšek and Jarošík 2005, Pyšek et al. 2009, Williamson et al. 2009, Wilson et al. 2007, de Albuquerque et al. 2011b).

Recent advances in biogeographical research indicate that the likelihood of biological invasions at the macro scale might be reasonably well predicted simply from knowledge of climatic condition and human-impact (Evans et al. 2005, Chown et al. 2005, Marini et al. 2009, Carboni et al. 2010, de Albuquerque et al. 2011a, 2011b). Human pressure and climate, however, do not influence the distribution of aliens equally, as demonstrated by de Albuquerque et al. (2011b) with the alien flora of Great Britain. Species with longer residence times in the invaded region are better adapted to climatic conditions, whereas recently arrived species depend more on human disturbances.

Several studies have also suggested that ecosystems or habitats differ considerably in the number of alien species they harbour (Vilà et al. 2007, Chytrý et al. 2008b, 2009, Pyšek et al. 2010), although not all native species are threatened by invaders and not all habitats are invaded. They have shown that habitat characteristics, in some contexts, may be even more important than propagule pressure and climatic factors (Chytrý et al. 2008a). To some extent, the effect of habitat integrates the effects of climate, geography (e.g. some habitats are typical of mountains, others of lowlands), and disturbance, because some habitats are more common in areas affected by humans, others in less disturbed areas with low human-impact.

Nonetheless, the patterns of distribution of species are determined by a combination of environmental and spatial processes. Therefore, to understand the determinants of variation in species richness it is important to disentangle the effects of environmental and spatial variables. Species distribution patterns are spatially structured for several

reasons: first of all, ecological processes are inherently spatial as they operate between neighbouring individuals; secondly species respond to variations in environmental factors, which are themselves spatially structured, thus inducing spatial dependencies in the distributions of species (Legendre 1993, Wagner and Fortin 2005).

In the present study, we analysed the spatial distribution of alien species in a region of north-eastern Italy characterised by high climatic and land-use heterogeneity to understand the influence of minimum residence time (MRT) on patterns of alien species richness. We conducted analyses with all alien plants occurring in the study region and within separate groups of alien species defined by residence time to evaluate the relative importance of climate, human pressure and landscape within the groups. More specifically, the primary objective was to quantify the relative role of environmental conditions and spatial patterns that could arise from niche-based processes such as environmental filtering (Weiher and Keddy 1999) and dispersal limitation (*sensu* Hubbell 2001), but also due to model misspecification (Dormann et al. 2007, Anderson et al. 2010, Kühn and Dormann 2012). A second objective was to assess whether residence time modifies responses of alien species richness to environmental variation (e.g. climate and human pressure) acting at a broader scale and to the processes acting at a finer scale (e.g. 'neutral processes' or unmeasured environmental variables). Considering environmental patterns, we hypothesised a direct influence of climate on the alien species having a longer residence time, indicating a key role of post-introduction environmental filtering ('climate filtering hypothesis'; Weiher and Keddy 1999) because these species have had a longer time to adjust their distributions to the climatic conditions (de Albuquerque et al. 2011b). Conversely, we hypothesised that introduction effort ('propagule pressure hypothesis'; Lockwood et al. 2005) and availability of new niches created by novel anthropogenic environmental conditions ('novel niche hypothesis'; Shea and Chesson 2002) have a consistent influence on the more recently arrived species.

Materials and methods

Study region

The study area was the Friuli Venezia Giulia region (north-eastern Italy), an area of 7845 km² (WGS84: N45°34.5'–46°38.3', E12°18.1'–13°55.1') on the southern border of the European Alps. About 43% of the territory is occupied by mountains, 38% by plains, and the remaining 19% by hills. The Adriatic coast extends for c.150 km, from the mouth of the Tagliamento River in the west to the Slovenian border in the east. The elevation ranges from sea level to 2780 m a.s.l. The local climates vary from sub-Mediterranean conditions in the southeast to alpine conditions in the inner valley. Differences in precipitation were mainly related to orographic effects. Northward, the highest precipitation occurs on the external alpine ridge, where the humid sea air is forced to

rise over the mountain range. The average annual rainfall varies from *c.* 1000 mm year⁻¹ along the Adriatic coast to *c.* 3000 mm year⁻¹ in the Julian Prealps. The annual mean temperature is 8.7°C and varies from 1.5°C in the Alps to 14.1°C along the Adriatic coast.

Data on alien plant species

Information on the distribution of vascular plants was extracted from the floristic atlas of the Friuli Venezia Giulia region (Poldini 2002). The region was subdivided into a regular grid of 81 cells or operational geographic units (OGUs, basic area: $\frac{1}{4}$ of a sheet of the IGM – Istituto geografico militare map of Italy – 1:50 000) each spanning 11 km × 13 km. Systematic data collection began in 1967, and the inventory was completed in 2007. Grid cells at the border of the region were included if more than 50% of their area lay within the region. This subdivision provided valid information of species occurrences for 57 grid cells. Only neophytes (introduced after the 15th century; see Pyšek et al. 2004) were classified as aliens in this study, following the checklist of Italian alien species (Celesti-Grapow et al. 2009) (Fig. 1). The size of the range of each species in the Friuli Venezia Giulia region was calculated as the proportion of mapping units occupied of the total available in the area.

The dates of the first records in the study region were assembled from the checklist of Italian alien species (Celesti-Grapow et al. 2009) and from the major floristic works and published floristic papers for the Friuli Venezia Giulia region (Marchesetti 1896–97, Pospichal 1897–99, Gortani and Gortani 1905–06, Poldini 1980, Mezzena 1986, Poldini 1991, 2002, 2009). We observed a pattern of species accumulation over time (Fig. 1a). Following de Albuquerque et al. (2011b), neophytes were grouped according

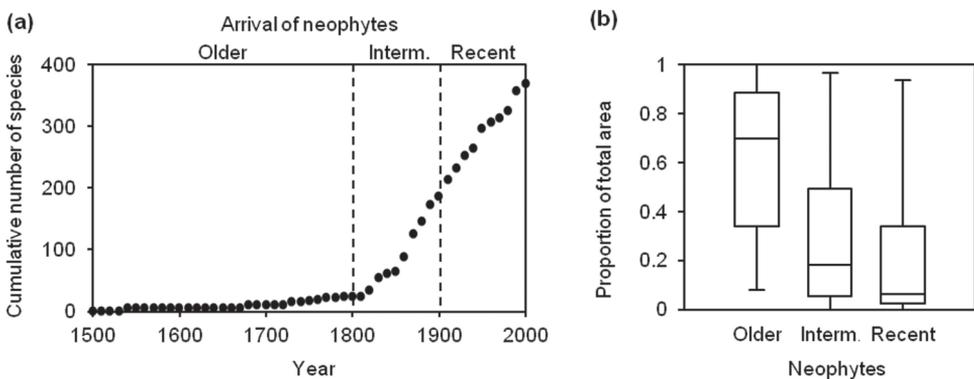


Figure 1. (a) Pattern of accumulation of neophyte species in the Friuli Venezia-Giulia region after the 15th century. Dashed lines divide the curve into three time periods used to differentiate three species groups: 1500–1800, a long period of gradual colonisation (older neophytes); 1800–1900, a first acceleration of the arrivals (intermediate neophytes); > 1900, a second acceleration (recent neophytes). (b) Box and whisker plots of the range sizes as a proportion of the total mapping units of three groups of neophytes differing in minimum residence time.

to three periods of arrival: (i) from 1500 until *c.* 1800, characterised by a slow increase in the number of alien species (23 species, about 6% of the total species number); (ii) from *c.* 1800 until *c.* 1900, characterised by an acceleration in the number of introductions (149 species, about 40% of the total species number); and (iii) from *c.* 1900 until the present, characterised by a further acceleration in the number of introductions (196 species, about 53% of the total species number). Hereafter, we refer to these groups as older neophytes (MRT = 200-500 years), intermediate neophytes (MRT = 100-200 years), and recent neophytes (MRT < 100 years).

Explanatory variables

We used the same grid to generate a series of explanatory variables quantifying climate, human-impact, and composition of the landscape. For each group we selected a first set of 35 variables of interest (Appendix S1).

For climatic variables, we considered annual precipitation (PREC) as an indicator of water availability and annual mean temperature (TEMP) as a measure of available energy. The data were retrieved from continuous raster-based climatic maps with a resolution of 100 × 100 m (1991–2008) provided by the Meteorological Observatory of Friuli Venezia Giulia (OSMER).

For variables of human-impact, we quantified population density for each cell (POP) using the dasymetric grid of population density disaggregated with Corine Land Cover and point survey data, as described in Gallego (2010), and obtained from the European Environment Agency (available at <http://www.eea.europa.eu/>). We measured the degree of urbanisation as the coverage of built-up areas (URB) (buildings, streets, and other artificial land uses) and as the total road length (ROAD) per cell. We also determined the proportion of agricultural land (AGR) as a further predictor of human disturbance. These land-cover variables were derived from the CORINE vector based map (2000), originally comprising 44 classes organized in three hierarchical levels (Servizio Sistema Informativo Territoriale e Cartografia - Direzione Centrale Pianificazione Territoriale, Autonomie Locali e Sicurezza, Trieste, Italy; available at <http://www.regione.fvg.it/>). These land-cover classes were obtained by merging the CORINE classes. To reduce collinearity, we only kept the ecologically most meaningful and least interrelated variables identified by calculating a Pearson's correlation matrix for model fitting. Variables related to human-impact were strongly interrelated ($r > 0.7$), so we used only population density as a proxy of the overall level of human pressure and urban development (Appendix S2).

The composition of the landscape was described by the distribution of natural and semi-natural habitats in each cell, excluding agricultural and artificial habitats used as a proxy of human disturbance. Percentages of habitats were calculated from the CORINE biotopes map (1:50 000) of the study region (Servizio Valutazione Impatto Ambientale - Direzione Centrale Ambiente e Lavori Pubblici, Trieste, Italy; available at <http://www.regione.fvg.it/>). A standard classification of European habitats from the

European Nature Information System (EUNIS; Davies et al., 2004, available at <http://eunis.eea.europa.eu/habitats.jsp>) was chosen as a convenient platform for evaluating biological invasions in Europe (Vilà et al. 2007, Chytrý et al. 2008b, Pyšek et al. 2010). CORINE biotopes were transferred to the EUNIS habitat classification based on the classification links of Lapresa et al. (2004). We used a total of 29 habitat classes. The 29 landscape variables (i.e. the proportion of each class in the cells) was reduced to 2 using a principal components analysis (PCA), and keeping the first two orthogonal axes. The broken-stick method was used as stopping rule in the PCA. This method only retains the components associated with eigenvalues larger than the values given by the broken-stick distribution, providing an accurate estimate of the dimensionality of the data (Jackson 1993). PCA analyses retained two axes that explained 33% of the variation in the composition of the landscape (Appendix S3). The first axis (called ALP) had the highest loadings to alpine habitats constrained by environmentally stressful conditions; the second axis (called TERM) had the highest loadings with thermophilous habitats constrained by dry and warm conditions. We thus used a total of five variables (temperature, precipitation, population density, alpine habitats, and thermophilous habitats) for model fitting.

Data analysis

Statistical analyses were performed with all alien plants occurring in the study region and within separate groups of alien species defined by residence time. First, the relative roles of climatic, human, and landscape variables on the observed variation in species richness were assessed using ordinary least squares (OLS) regression. Given that we had no a priori hypotheses supporting interactions between the considered explanatory variables, we did not include any interaction terms in our model selection procedure. We performed a backward manual deletion procedure starting from the full model ($P < 0.05$). We standardised the response variables (i.e. species richness) to zero mean and unit standard deviation to make the parameter estimates comparable. To produce a set of non-negative standardised variables, a constant value of three was added to all values.

Second, we evaluated the robustness of the standardised regression coefficients of our non-spatial OLS models by comparing them with those of spatial models generated with spatial eigenvector mapping (SEVM) techniques (Borcard and Legendre 2002). Because autocorrelation usually causes further variables to become insignificant, but not insignificant variables to become significant, the backward-selected variables in OLS models were entered into the SEVM (Kühn 2007). The selection of the eigenvectors was implemented with a forward method that focused on minimising residual autocorrelation (Griffith and Peres-Neto 2006). A Gabriel connection was used to describe the spatial relationships between spatial units (Legendre and Legendre 1998). Gabriel networks approximate the rook scheme when the data are in a regular grid (as in our case). Using these short-distance connections is preferable (i.e. more

conservative; Griffith 1996) to using inverse-decaying distances, since in most empirical data sets, residual spatial autocorrelation tends to be stronger at smaller distance classes (Bini et al. 2009). The spatial analyses were performed using Spatial Analysis in Macroecology (SAM) software (version 4.0; Rangel et al. 2010).

Third, we used hierarchical partitioning (HP) (Chevan and Sutherland 1991) to rank the importance of each variable most likely to affect variation in species richness. HP is an analytical method of multiple regression that quantifies the relative importance of each explanatory variable to the total explained variance of the regression model, both independently and together with the other explanatory variables, calculated for all possible candidate models. HP was conducted using the 'hier.part' package implemented in R version 2.14.1 (R Development Core Team 2011). The independent effects were tested using a randomization routine ($n = 200$), which gives Z -scores for the generated distribution of randomized independent contributions and a level of statistical significance (P) based on this score. We used a normal error distribution and R^2 as a measure of goodness-of-fit. We then used the results of HP to compare the relative importance of each group of environmental variables (i.e. climate, human-impact, and landscape). Environmental sets were assembled using the percentages of independent effects derived by HP. The climatic set was assembled using the independent effects of temperature and precipitation, the human-impact set using the independent effect of population density, and the landscape set using the independent effects of alpine and thermophilous habitats.

Finally, we conducted partial regressions to partition the variation explained by environmental (backward-selected climatic, human-impact, and landscape variables) and spatial variables into independent and covarying components. The total explained variation in species richness was partitioned into three components (Borcard et al. 1992, Peres-Neto and Legendre 2010): (i) the non-spatial environmental variation (E), which is the fraction of the variation in species richness that can be explained by the environmental descriptors independently of any spatial structure; (ii) the spatial structuring in the species data that is shared by the environmental data ($E \cap S$), i.e. induced spatial dependence, which is defined as the spatial structure of the response variables that exists due to the spatial structure of the explanatory variables; and (iii) the spatial patterns (S) in the species data that are not shared by the environmental data, due to large scale spatial trends and small scale autocorrelation generated by the species themselves (i.e. from biotic processes such as growth, dispersal, and mortality) or simply due to model misspecification (i.e. ignoring an important variable of spatial structure or falsely assuming a linear relationship that indeed is non-linear; Dormann et al. 2007, Anderson et al. 2010, Kühn and Dormann 2012). The variation explained in each model was reported as the adjusted R^2 (R^2_{adj}), which takes the number of predictor variables and sample size into account to prevent the inflation of R^2 values (Peres-Neto et al. 2006). When a negative R^2_{adj} was obtained, we interpreted it as a zero value (Peres-Neto et al. 2006), which means that not all fractions of one variation partitioning always add up to a perfect 100%. The partial regression analyses were implemented in the 'vegan' package for R.

Results

Range sizes decreased significantly from older to intermediate to recent neophytes (Fig. 1b, one-way ANOVA, $F = 7.73$, $P < 0.001$).

The multiple regression models showed several differences in the significance and rank of the standardised regression coefficients among separate groups of alien species defined by residence time (Table 1). For older neophytes, the model retained temperature and population density. For intermediate and recent neophytes, the models included temperature, population density, and thermophilous habitats. Temperature was the most important factor and was positively correlated with alien species richness in all the models. Positive partial coefficients were also found for the regression coefficients of the population density, but they were always substantially lower than those of temperature, especially for older neophytes. Thermophilous habitats had a weaker positive relationship with alien species in the models of intermediate and recent neophytes, suggesting that alien species richness is higher in dry and warm conditions.

Including spatial filters in the model increased the amount of variance able to be explained, while the rank of the standardised regression coefficients of the variables was similar to OLS in all but the models for recent neophytes (Table 1). The effects of spatial filters on the P -values of the regression coefficients, however, were more important.

Table 1. Minimum adequate models for the relationships between alien species richness and the predictors (TEMP, annual mean temperature; POP, population density; TERM, thermophilous habitats) in the Friuli Venezia-Giulia region (Southern Alps) tested in the ordinary least squares (OLS) multiple regression models and in the spatial models generated with spatial eigenvector mapping (SEVM) techniques. The models are for (a) all neophyte plants occurring in the study region and within separate groups of neophytes defined by minimum residence time (MRT): (b) older neophytes (MRT = 200-500 yr), (c) intermediate neophytes (MRT = 100-200 yr), and (d) recent neophytes (MRT = <100 yr). Standardized regression coefficients (β) are presented.

	OLS				SEVM			
	β	t	P	R^2	β	t	P	R^2
(a) All neophytes				0.82				0.85
TEMP	0.558	7.895	<0.001		0.815	3.543	0.001	
POP	0.444	6.293	<0.001		0.406	4.724	<0.001	
TERM	0.209	3.610	<0.001		0.207	2.740	0.009	
(b) Older neophytes				0.66				0.82
TEMP	0.685	7.033	<0.001		0.705	4.735	<0.001	
POP	0.196	2.012	0.049		0.395	4.747	<0.001	
(c) Intermediate neophytes				0.81				0.90
TEMP	0.557	7.710	<0.001		0.812	3.987	<0.001	
POP	0.447	6.187	<0.001		0.319	4.329	<0.001	
TERM	0.177	2.995	0.004		0.028	0.390	0.699	
(d) Recent neophytes				0.72				0.84
TEMP	0.471	5.343	<0.001		0.246	1.478	0.147	
POP	0.461	5.227	<0.001		0.356	4.347	0.001	
TERM	0.237	3.283	0.002		0.137	1.828	0.075	

Temperature was significant in the OLS model of recent neophytes but insignificant in the SEVM model and the thermophilous habitats in the models of intermediate and recent neophytes. Moreover, the standardised regression coefficients of predictors showed strong differences between the OLS and SEVM models. Temperature (e.g. for all and intermediate neophytes) and population density (e.g. for older neophytes) particularly had strong shifts in coefficients between the OLS and SEVM analyses (Table 1).

The results of hierarchical partitioning generally reflected those yielded by the regression models but produced slightly different results concerning the relative importance of some variables (Fig. 2a). The variable ranking indicated that both temperature and alpine habitats, followed by population density and precipitation, were the best predictors, with the highest independent effect on the number of alien species for older neophytes. For intermediate and recent neophytes, population density showed the largest independent effects followed by temperature. Considering the three sets of environmental variables (Fig. 2b), there was a reduction in the influence of climate and landscape, and an increase in the influence of human-impact, from the older neophytes to the recent neophytes (from 49% to 35% for climate, from 34% to 29% for landscape, and from 17% to 36% for human-impact).

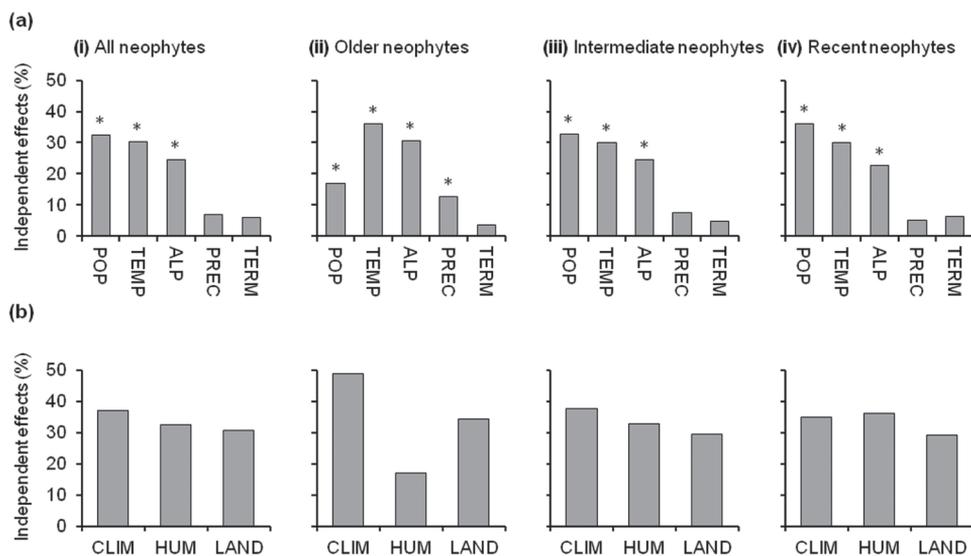


Figure 2. The relative independent effects of (a) environmental variables and (b) environmental variable sets (CLIM-climate, HUM-human-impact, and LAND-landscape) on species richness of (i) all neophytes, (ii) older neophytes, (iii) intermediate neophytes, and (iv) recent neophytes. Variables are ranked by the size of the independent effect in model (i) (i.e. all neophytes). Asterisks indicate statistical significance ($P < 0.05$) of the independent effect of each variable based on randomization tests ($n = 200$). POP, population density; TEMP, annual mean temperature; ALP, alpine habitats; PREC, annual precipitation; TERM, thermophilous habitats. Climate includes temperature and precipitation; human-impact includes population density; and landscape includes alpine and thermophilous habitats.

Partial regression analyses showed that most of the variation explained by the models is attributable to spatially structured environmental variation ($E \cap S$, *c.* 70–80%), while environment (E) and space (S) each had small independent effects (*c.* 10–20%) (Fig. 3). The independent environmental effect (E), however, decreased and conversely the independent spatial effect (S) increased, from the older neophytes to the recent neophytes (from 14% to 9% for environment and from 11% to 18% for space).

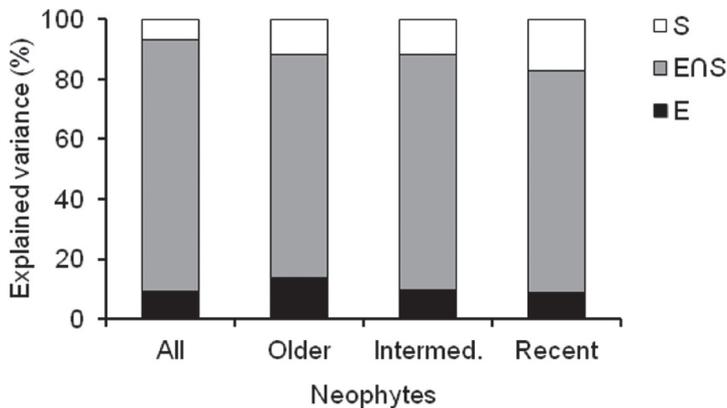


Figure 3. Variation partitioning of alien species richness per cell into environment and space for all neophytes, older neophytes, intermediate neophytes, and recent neophytes. The total variation explained was split into nonspatial environmental variation (**E**), spatially structured environmental variation (**ENS**), and spatial structure not explained by the environmental variables (**S**). Results are expressed as part of the total explained variation for each component (i.e. values add up to 100%).

Discussion

The observed tendency for the sizes of the ranges of alien species to increase with minimum residence time in the Friuli Venezia Giulia region is consistent with patterns reported elsewhere in the world (Rejmánek 2000, Castro et al. 2005, Pyšek and Jarošík 2005, Ahern et al. 2010, de Albuquerque et al. 2011b). In general, the longer the time that a species is present in a region, the more it extends into its potential range because it has had more opportunities to be introduced several times at various locations and more time to disperse naturally, in order to overcome a ‘lag’ period (Kowarik 1995, Sakai et al. 2001, Gassó et al. 2012). Furthermore, residence time is also related to propagule pressure (Richardson and Pyšek 2006), i.e. alien species with longer residence times tend to produce and disperse more propagules.

This study also contributes to an understanding of how environmental-filtering (Weiher and Keddy 1999) and biotic processes, such as dispersal limitation (Svenning and Skov 2004), contribute to the distribution of alien species richness. We used environmental variables that summarise climatic conditions and human pressure to assess the role of environmental and disturbance filters for explaining patterns of alien species richness at a broader scale. Spatial patterns modelled by spatial

eigenvector mapping were used to infer non-modelled processes acting at finer scales, such as dispersal and biotic interactions (Borcard et al. 2004) but also environmental factors that were not included in the analysis (Borcard et al. 2004, Dorman et al. 2007, Anderson et al. 2010).

Incorporating spatial filters in the models highlights several differences in the parameter estimates and shifts in coefficients between non-spatial and spatial regressions. These effects have also been observed in other studies (e.g. Dormann 2007, Kühn 2007) and confirm the misestimation of the relationships between richness and its potential drivers when considering only non-spatial models. Why and when such shifts in coefficients between spatial and non-spatial methods occur, however, still remain largely unclear, and a recent study failed to support this or any other explanation for such shifts (Bini et al. 2009).

Role of environmental conditions

Environmental variables ($E + E \cap S$) account for *c.* 80–90% of the explained variation in alien species richness, although 70–75% of this variation is spatially-structured ($E \cap S$). The shared variation between explanatory variables and spatial descriptors is produced by induced spatial dependence (Peres-Neto and Legendre 2010), suggesting that environmental conditions are particularly important in determining alien species richness. As suggested by Cottenie (2005), including variables quantifying spatial structure should greatly improve the understanding of the factors that structure communities.

Specifically, we found strong differences in the importance of temperature and human disturbance on patterns of species richness in the three residence time groups. The association of alien species richness with temperature is stronger for groups with species having a longer residence time, which declines to become not significant for more recently arrived species, as shown in the spatial models. Furthermore, hierarchical partitioning shows a reduction in the influence of climate, from the older neophytes to the recent neophytes. These results confirm the hypothesis that climate filtering (Weiher and Keddy 1999) has a key role in the post-introduction of alien species, because these species have had a longer time to adjust their distributions to the climatic conditions. The longer the residence time is, the greater the possibility for a species to have fully occupied its potential range in the study area (de Albuquerque et al. 2011b, Gassó et al. 2012). Conversely, human pressure has a greater influence on species that arrived more recently, although its influence remains significant in all groups. This confirms that human pressures are key drivers of alien plant invasions. Anthropogenic changes to the landscape create niche opportunities for invaders, as pointed out in the ‘novel niche hypothesis’ (Shea and Chesson 2002). For example, abandoned fields and urban areas host a number of exotic species that cannot survive in adjacent forests (Lonsdale 1999). In addition, human pressure can shape the early stage of introduction, when humans determine the number of species introduced into a region and the number of at-

tempts of introduction and/or number of individuals of each species introduced ('propagule pressure hypothesis'; Lockwood et al. 2005).

Landscape variables instead show a trend similar to that observed for climate, with a reduction in the influence from the older neophytes to the recent neophytes. This trend could be due to the covariation among climatic and landscape variables. We particularly found a strong negative correlation between temperature and alpine habitats ($r = -0.97$; $P < 0.001$) and a meaningful negative correlation between precipitation and thermophilous habitats ($r = -0.56$; $P < 0.001$) (Appendix S2).

Role of spatial patterns

The shared variation between spatial descriptors and environmental variables is likely acting at broader spatial scales, accounting for environmental heterogeneity (e.g. climate and human pressures) and induced spatial dependence, whereas dispersal and biotic interactions such as competition, mortality, and social organisation are likely acting at finer spatial scales (Borcard et al. 2004, Cottenie 2005). We found an increasing fraction of the alien species richness explained solely by spatial descriptors (S) from the older neophytes (*c.*11%) to the recent neophytes (*c.*18%). This result suggests that fine-scale spatial patterns (those that can cause spatial autocorrelation), generated by dynamic processes regulating species richness, are important factors in determining alien plant invasions. Although these interpretations seem reasonable, recent discussions on the subject indicate that caution is required when attributing components of diversity to the outcome of these processes (Anderson et al. 2010, Smith and Lundholdm 2010, De Cáceres in press). The effect of environmental control and dispersal processes may be confounded by the frequent correlation between the spatial patterns created by dispersal limitation and the spatial arrangement of the environment (Smith and Lundholdm 2010, Anderson et al. 2010). Moreover, apparent spatial portions interpreted as 'neutral' could simply have been due to unmeasured environmental variables (Dormann et al. 2007, Anderson et al. 2010).

Conclusions

This study confirms the importance of considering residence time when studying spatial patterns of alien species richness and identifies residence time as a pivotal factor in the current distribution of alien species, i.e. climatic factors being most important for species with a longer residence time and factors related to human populations and habitat identity for species with shorter residence time. Our results also contribute to a better understanding of the influence of climate and human pressure on alien species richness and how these drivers shift their influences during the process of invasion. Additionally, the inclusion of spatial descriptors is important for explaining patterns of alien species and for unravelling the role of spatial autocorrelation generated by biotic processes.

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Appendix S1

List of all predictors considered.

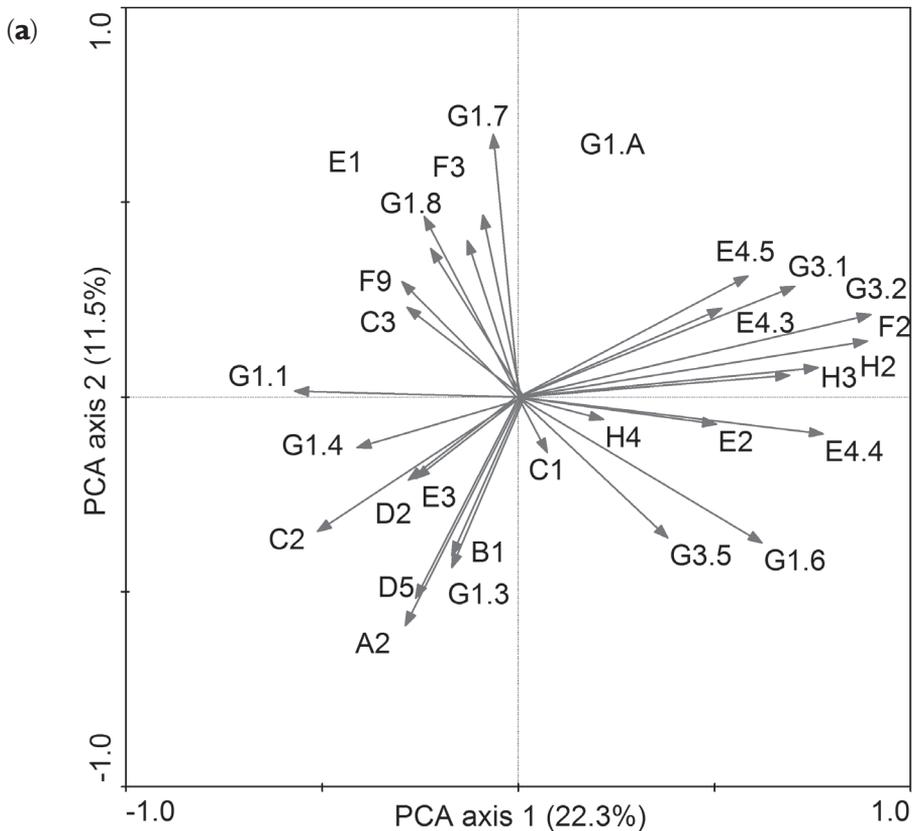
Variable names and explanation		Unit	Mean ± SD	Min	Max
Climate					
PREC	Annual precipitation	mm	1551 ± 365	976	2465
TEMP	Annual mean temperature	°C	10.18 ± 2.86	4.75	13.68
Human-impact					
POP	Population density	per km ²	126.5 ± 158.5	4.10	831.80
ROAD	Road length	km	99.37 ± 61.90	3.88	258.70
URB	Total area covered by built-up elements	%	7.30 ± 7.31	0.07	32.45
AGR	Area covered by agricultural area	%	34.54 ± 34.06	0.00	89.55
Land-use					
A2	Littoral sediment	%	1.73 ± 7.91	0.00	50.17
B1	Coastal dunes and sandy shores	%	0.01 ± 0.08	0.00	0.57
C1	Surface standing waters	%	0.13 ± 0.28	0.00	1.52
C2	Surface running waters	%	0.25 ± 0.45	0.00	1.97
C3	Littoral zone of inland surface waterbodies	%	1.65 ± 2.03	0.00	8.60
D2	Valley mires, poor fens and transition mires	%	0.03 ± 0.11	0.00	0.53
D5	Sedge and reedbeds	%	0.12 ± 0.61	0.00	4.26
E1	Dry grasslands	%	2.20 ± 4.35	0.00	21.73
E2	Mesic grasslands	%	1.60 ± 1.63	0.00	8.77
E3	Seasonally wet and wet grasslands	%	0.01 ± 0.03	0.00	0.17
E4.3	Acid alpine and subalpine grassland	%	0.64 ± 1.74	0.00	10.36
E4.4	Calcareous alpine and subalpine grassland	%	1.81 ± 2.68	0.00	10.14
E4.5	Alpine and subalpine enriched grassland	%	0.15 ± 0.42	0.00	1.86
F2	Arctic, alpine and subalpine scrub	%	4.47 ± 7.15	0.00	31.88
F3	Temperate and mediterranean-montane scrub	%	1.75 ± 3.49	0.01	21.39
F9	Riverine and fen scrubs	%	0.12 ± 0.27	0.00	1.47
G1.1	Riparian and gallery woodland	%	0.67 ± 0.97	0.00	3.37
G1.3	Mediterranean riparian woodland	%	0.00 ± 0.01	0.00	0.10
G1.4	Broadleaved swamp woodland not on acid peat	%	0.08 ± 0.22	0.00	1.05
G1.6	[<i>Fagus</i>] woodland	%	13.69 ± 16.63	0.00	55.24
G1.7	Thermophilous deciduous woodland	%	6.53 ± 9.51	0.00	46.33
G1.8	Acidophilous [<i>Quercus</i>]-dominated woodland	%	2.31 ± 5.66	0.00	27.32
G1.A	Meso- and eutrophic [<i>Quercus</i>] and related woodland	%	1.78 ± 4.00	0.00	20.71
G3.1	[<i>Abies</i>] and [<i>Picea</i>] woodland	%	7.59 ± 15.17	0.00	55.17
G3.2	Alpine [<i>Larix</i>] - [<i>Pinus cembra</i>] woodland	%	1.42 ± 2.33	0.00	8.57
G3.5	[<i>Pinus nigra</i>] woodland	%	3.88 ± 6.47	0.00	23.82
H2	Screes	%	0.75 ± 1.31	0.00	6.84
H3	Inland cliffs, rock pavements and outcrops	%	1.37 ± 2.42	0.00	9.97
H4	Snow or ice-dominated habitats	%	0.02 ± 0.15	0.00	1.13

Appendix S2

Pearson correlations between explanatory variables (TEMP, annual mean temperature; PREC, annual precipitation; POP, population density; ROAD, road length; URB, total area covered by built-up elements; AGR, area covered by agricultural area; ALP, alpine habitats; TERM, thermophilous habitats).

	TEMP	PREC	POP	ROAD	URB	AGR	ALP
PREC	-0.563						
POP	0.574	-0.412					
ROAD	0.721	-0.325	0.744				
URB	0.711	-0.505	0.916	0.827			
AGR	0.843	-0.699	0.498	0.651	0.724		
ALP	-0.971	0.482	-0.512	-0.654	-0.631	-0.754	
TERM	0.045	-0.556	0.004	-0.079	0.088	0.306	0.000

Appendix S3



(a) Principal components analysis (PCA) diagram of 29 habitat classes, for codes of habitat classes please see Appendix S1

(b) Eigenvector scores of 29 habitat classes in two main PCA axes. Values are ranked in order of absolute magnitude along PCA 1. The five highest absolute eigenvector scores for each PCA axis are indicated in bold. Values in parentheses indicate variance accounted for by each axis.

Habitat		PCA1 - ALP (22.3%)	PCA2 - TERM (11.5%)
G3.2	Alpine [<i>Larix</i>] - [<i>Pinus cembra</i>] woodland	0.899	-0.211
F2	Arctic, alpine and subalpine scrub	0.890	-0.143
E4.4	Calcareous alpine and subalpine grassland	0.777	0.094
H2	Scree	0.764	-0.075
G3.1	[<i>Abies</i>] and [<i>Picea</i>] woodland	0.704	-0.284
H3	Inland cliffs, rock pavements and outcrops	0.691	-0.056
G1.6	[<i>Fagus</i>] woodland	0.621	0.374
E4.5	Alpine and subalpine enriched grassland	0.585	-0.311
G1.1	Riparian and gallery woodland	-0.570	-0.015
E4.3	Acid alpine and subalpine grassland	0.518	-0.227
E2	Mesic grasslands	0.505	0.070
C2	Surface running waters	-0.492	-0.349
G3.5	[<i>Pinus nigra</i>] woodland	0.381	0.361
G1.4	Broadleaved swamp woodland not on acid peat	-0.322	-0.095
A2	Littoral sediment	-0.282	-0.644
E3	Seasonally wet and wet grasslands	-0.268	-0.200
D5	Sedge and reedbeds	-0.262	-0.600
E1	Dry grasslands	-0.260	0.494
D2	Valley mires, poor fens and transition mires	-0.249	-0.188
F9	Riverine and fen scrubs	-0.238	0.246
H4	Snow or ice-dominated habitats	0.217	0.058
C3	Littoral zone of inland surface waterbodies	-0.195	0.187
B1	Coastal dunes and sandy shores	-0.189	-0.462
G1.A	Meso- and eutrophic [<i>Quercus</i>] and related woodland	-0.187	0.330
G1.3	Mediterranean riparian woodland	-0.182	-0.464
G1.8	Acidophilous [<i>Quercus</i>]-dominated woodland	-0.138	0.421
F3	Temperate and mediterranean-montane scrub	-0.085	0.484
C1	Surface standing waters	0.073	0.142
G1.7	Thermophilous deciduous woodland	-0.040	0.672

Tolerance to air exposure of the New Zealand mudsnail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca) as a prerequisite to survival in overland translocations

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Abstract

Spreading throughout a new ecosystem is the last step of an exotic species to become invasive. In the case of invasive aquatic molluscs, tolerance to air exposure is one of the main mechanisms allowing overland translocation and spreading. The mudsnail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca) is native to New Zealand but it has spread worldwide, invading ecosystems in Europe, Australia, America and Asia. The aim of our study is to assess mudsnail tolerance to air exposure, which may contribute to the successful overland translocation of this species. We conducted a laboratory experiment with four levels of air exposure (9, 18, 24 and 36 hours in a controlled climatic chamber). Snails were placed for 60 seconds in a laboratory paper filter to remove surface snail water. Then they were placed back in empty vessels during the four periods of air exposure, except the control group, which was immediately returned to water. At the end of each period of air exposure all vessels were filled with water and the cumulative mortality was monitored after 24, 96, 168 and 264 hours of rehydration. The calculated Lethal Times (i. e. the time of air exposure (in hours) necessary to cause the death of 50% (LT50) or 99% (LT99) of the population) and their 95% confidence limits at 24, 96, 168 and 264 hours were 28.1 (25.2–31.9), 26.9 (24.2–30.1), 25.9 (23.4–28.9) and 25.9 (23.4–28.9) hours, respectively for LT50, and 49.6 (42.7–63.3), 45.6 (39.9–56.5), 43.2 (38.0–53.0) and 43.2 (38.0–53.0) hours, respectively for LT99. Therefore an air exposure time over 43 hours caused the death of all studied individuals during all monitoring periods. Extending the monitoring period beyond 24 hours did not significantly change lethal times. Therefore, we recommend exposing fishing tools or boats at open air during at least 53 hours as a low cost measure to control mudsnail spread in early stages of invasion.

Keywords

Exotic species, aquatic ecosystem, mudsnail, desiccation, spread

Introduction

Biological invasions are one of the most important ecological problems at worldwide scale (Everett 2000). Humans have overcome all biogeographical barriers, connecting all parts of the World (Everett 2000, Nentwing 2007). This fact has facilitated the spread of exotic species on a large scale, causing a decline of biodiversity, a biotic homogenization of ecosystems (Enserink 1999, Gherardi 2007, Nentwing 2007), and huge economic costs (Everett 2000). The process of becoming an invasive species (i.e. an widespread exotic species that causes adverse effects on the invaded ecosystem) (Davis and Thompson 2000; Mack et al. 2000) is very complex and it is governed by an interaction of habitat conditions (e.g., climate), species functional traits (e.g., tolerance to environmental factors) and human factors (e.g., uses of the species) (Kolar and Lodge 2001, Gherardi 2007, Nentwing 2007, Castro-Díez et al. 2011). The invasion process includes three main steps: transport, establishment and spread (Kolar and Lodge 2001, Sakai et al. 2001). Exotic species must overcome all these steps through a combination of several mechanisms. Therefore only a small proportion of the exotic species arriving in a new ecosystem becomes invasive (Williamson and Fitter 1996, Sakai et al. 2001).

A rapid spread is the previous step to consider an exotic species as invasive. In the case of aquatic molluscs, tolerance to air exposure (i.e., absence of contact with water during a period of time) is one of the main prerequisites allowing overland transport, because many vectors require the ability to survive out of water (e.g., attached to nets, boats, buoys, waterfowl, etc.) (Ricciardi et al. 1995, Richards et al. 2004). Therefore the longer the period the mollusc can survive out of water the higher is the probability of successful translocation.

The mudsnail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca) is native to New Zealand but has spread worldwide, invading ecosystems in Europe, Australia, America and Asia (Alonso and Castro-Díez 2008). This snail is called mudsnail since it buries itself in the sediment during drought or cold periods (Duft et al. 2003a). This mollusc has a solid and well-formed operculum, a small narrow shell (length 1–10 mm), and it possesses a strong mobile and retractile foot (Winterbourn 1970, Kabat and Hershler 1993, Duft et al. 2003a, b). Non-native populations are parthenogenetic with almost all individuals being females, which allows a rapid colonization of the invaded ecosystems (Alonso and Castro-Díez 2008). Additionally, the potential number of generations per year is up to six, and the average number of offspring per adult is 230 (Møller et al. 1994, Richards 2002). This species can spread through transport vectors, such as fish, waterfowl or fishing tools, due to its ability to survive the passage through the digestive tract (in the case of fish) or to its air exposure tolerance (in the case of waterfowl or fishing tools) (Richards et al. 2004, Alonso and Castro-Díez 2008, Vinson and Baker 2008). The distance covered by means of the latter mechanism depends on

how much time the mudsnail can survive out of water, which has not received much attention so far (but see Richards et al. 2004, Lysne and Koetsier 2006). Additionally, the knowledge of the air exposure tolerance can help to establish protocols to avoid translocation, as air exposure is a simple and low-cost method for exotic species control (Richards et al. 2004). This knowledge is crucial to avoid the establishment of mudsnail in new aquatic ecosystems (Richards et al. 2004) as the air-exposed vectors (e.g. birds, wild animals, human vectors, etc.) can be used by mudsnail to invade new aquatic ecosystems or to spread in an invaded ecosystem.

Previous studies on aquatic invasive molluscs have shown contrasting tolerances to air exposure among different species and environmental conditions. In the case of the mudsnail there are two studies for American populations (Richards et al. 2004, Lysne and Koetsier 2006). Both studies showed higher tolerance in bigger individuals and longer survival in damp and moist environments than in dry ones. However, no information is available on the air exposure tolerance of European populations of mudsnail which could possess contrasting tolerances. The aim of this study is the assessment of the air exposure tolerance of mudsnail under laboratory conditions to different periods of water absence to make recommendations to control the spread of this exotic species.

Material and methods

The snails used in the experiment (mean length size of 3.8 mm, total number of 120) were obtained from our laboratory culture at the Department of Ecology (University of Alcalá). The culture was initiated with snails collected from the natural unpolluted upper reach of the Henares River (Guadalajara, Spain) in January 2009. Snails were kept in 60L glass aquaria with USEPA moderately hard water (96 mg NaHCO_3 , 60 mg $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 4 mg KCl, 122.2 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ per litre of deionised water) (US Environmental Protection Agency 2002), enriched with calcium carbonate (10 mg CaCO_3 per litre of deionised water). The culture was kept at room temperature (20–22°C) with natural photoperiod (minimum daylight=9 h and maximum daylight=15 h). Stock snails were fed with fish food (Tetra© GmbH, Germany) and dry Spirulina (Sera© Spirulina Tabs). The dry food per individual and day was around 0.10 mg. The culture was aerated with an air pump and an aquarium filter. Under these conditions snails reproduce and grow.

Snails for the experiment were obtained from the culture and acclimatized to the experimental conditions (15°C air temperature) in a controlled climatic chamber (Ansonic VSC3207). Experimental water was the same as that in the culture aquaria. Snails were acclimatized over seven days before the experiment. During this period snails were normally fed. This temperature was selected as the average for spring conditions, as higher temperatures decrease their survival (Richards et al. 2004).

An air exposure experiment was conducted, using four different periods (0, 9, 18, 24 and 36 hours without water in a controlled climatic chamber), each in triplicate. Eight adult snails were used in each replicate (120 individuals altogether), which consisted of a glass vessel of 8 cm diameter and 6 cm height. Snails of each replicate and air exposure

treatment were taken with forceps and placed for 60 seconds on a laboratory paper filter (Anoia Filter, 73g/m²), rolling them over the paper until no surface water was visible on their surface (i.e., snail shell without shine). After this preliminary dehydration, snails were placed in empty vessels for 9, 18, 24 or 36 hours, depending on the assigned treatment. Three additional replicates (with 8 snails each) were returned to the water as control treatment (0 hours of air exposure with preliminary dehydration). At the end of each air exposure period, vessels from each treatment were filled with control water (US-EPA water) and the cumulative mortality was monitored after 24, 96, 168 and 264 hours of hydration, including control treatment. Mortality was assessed by observing each snail under a binocular. An animal with a closed operculum was considered immobile and alive if after touching the operculum with forceps the snail retracted its soft body. If not it was considered dead. During the experiment air temperature and humidity in the climatic chamber were measured using a digital thermometer-hydrometer.

The lethal time 50 (LT50) and 99 (LT99) (i. e., the time of air exposure in hours that caused the death of 50% and 99% of the studied population, respectively) after 24, 96, 168 and 264 hours since the end of air exposure, and their respective 95% confidence limits were calculated using probit regression analysis (Finney 1971). The dependent variable was the probit transformation of the proportion of dead snails responding to each air exposure period (9, 18, 24 and 36 hours without water), and the independent variable was the air exposure period. Statistical analyses were conducted with SPSS 15.0 software. LT values were compared between different monitoring times using the confidence interval overlap test (Wheeler et al. 2006)

Results

The mean±SD (n=10) environmental conditions during the experiments were 68.9±6.6 % of relative air humidity and 15.4±0.5 °C of air temperature. The mortality of *P. antipodarum* was relatively low up to 20 hours of air exposure. After this time, mortality increased (Fig. 1). Air exposure times between 25.9 and 28.1 (LT50), and between 43.2 and 49.6 hours (LT99) caused the death of the half and all studied population, respectively (Table 1). An increase of the monitoring time neither changed LT50 nor LT99 values, as the confidence limits between different monitoring times overlapped (Confidence interval overlap test; p>0.05). Therefore, an air exposure treatment of 53 hours (upper limit of LT99 at 264 hours) assured the death of all snails.

Table 1. LT50 and LT99 values (in hours) at 24, 96, 168 and 264 hours for each time of mortality monitoring. 95% confidence limits are presented in parentheses.

	24 h	96 h	168 h	264 h
LT50 (in hours)	28.1 (25.2–31.9)	26.9 (24.2–30.1)	25.9 (23.4–28.9)	25.9 (23.4–28.9)
LT99 (in hours)	49.6 (42.7–63.3)	45.6 (39.9–56.5)	43.2 (38.0–53.0)	43.2 (38.0–53.0)

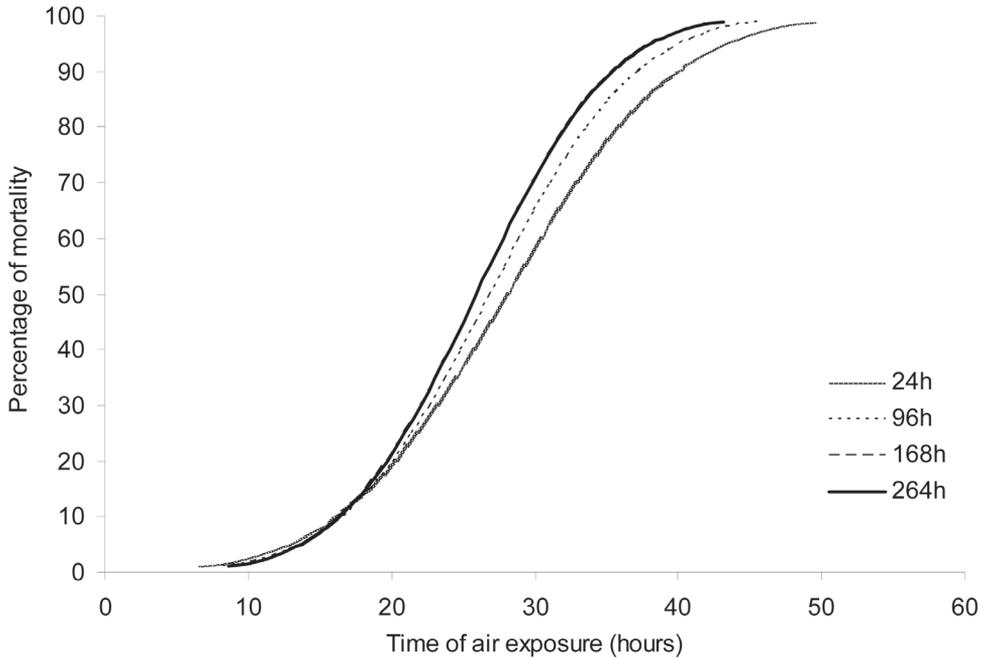


Figure 1. Modelled probit curves for the percentage of mortality of *Potamopyrgus antipodarum* for each time of monitoring after returning the animals to water. Curves at 168 hours and 264 hours fully overlapped.

Discussion

Our study demonstrated that survival of air-exposed mudsnail over short-period transport (<24 h) through non-aquatic media is highly probable. This scenario could be accomplished in waterfowl movements, transport of fishing tools (rubber boots, boats, nets, etc.) or movement of terrestrial animals (sheep, cows, domestic dogs, deer, etc.). Therefore the desiccation tolerance may be one of the most important traits promoting the translocation of mudsnail in inland waters. In an American population Richards et al. (2004) found a very similar tolerance of mudsnail to air exposure, with a 99% lethal time of 50 hours for individuals with shell size and environmental conditions similar to those in our study. Additionally, these authors found that under favourable conditions (temperature 9°C) 99% of the population died after 60 hours, and that small individuals were less tolerant than adults. This result is in the range of the upper LT99 confidence limit found in our study. Our study was conducted under relatively high humidity (mean RH of 69%) and 15°C of temperature which represent favourable conditions to tolerate air exposure in molluscs (Byrne et al. 1988, Alli and Nakamura 1999, Richards et al. 2004). Therefore, unfavourable conditions (high temperature, dry summer, low humidity during transport, etc.) would shorten this LT99. In fact, other studies with molluscs have shown that air exposure under lower relative humidity and/or higher temperature cause mortality in shorter times (Byrne et al. 1988, Ali and Nakamura 1999, Richards et al. 2004, Havel 2011). Additionally, most of these studies showed that smaller snails

were more sensitive to air exposure than larger snails. Therefore our recommended air exposure of 53 hours, calculated for large individuals and favourable conditions, could be time enough to ensure no survival of mudsnail in non-water translocation. Besides, large snails (the most prone to tolerate air transport) are the easiest to locate and remove from macrophytes, boat surface or waders. Other transportation vectors, such as domestic animals and waterfowl are risky vectors as the time to reach a new water body can be relatively short.

Air exposure caused several harmful effects in aquatic animals. In the case of mollusc a rapid loss of body water has been observed, being faster in small individuals than in large individuals (Ricciardi et al. 1995). However, other physiological effects of air exposure have been reported for molluscs, such as depletion of energy reserves, acidosis and accumulation of anaerobic metabolites (Byrne et al. 1990). Therefore, one of those effects (or a combination of them) can cause the mortality observed in our experiment.

The hard operculum of New Zealand mudsnail can contribute to explain its high tolerance to air exposure. In a previous study, Wood et al. (2011) showed that adults of the native non operculated gastropod *Physa gyrina* presented a lower tolerance to air exposure than the operculated invasive snail *Bithynia tentaculata*. The hard carbonate operculum of mudsnail is a good barrier to avoid water loss, which can prolong the tolerance to air exposure, as shown for similar species (Wood et al. 2011).

Conclusions

Avoiding the spread of invasive species is crucial for the conservation of aquatic native biodiversity. The results of our study suggest several low-cost measures to decrease the spread of mudsnail among reaches and aquatic ecosystems. First we recommend a desiccation treatment to all tools or instruments that are going to be repeatedly used in different aquatic ecosystems (e.g., exposing fishing tools at air during at least 50 hours, preferably at full sun light). Second, we recommend avoiding the access of wild and domestic animals to infected reaches or lakes (e.g., by using physical barriers or scarecrows for waterfowl). These simple measures may reduce mudsnail translocations in early stages of invasion.

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