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Identifying thresholds and ceilings in plant community recovery for optimal management of widespread weeds

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Abstract

A substantial body of work underlies the theory and practice of early intervention in the management of invasive alien plants, but less attention has been paid to the strategic management of widespread weeds, especially in the context of natural asset recovery. The assumption lingers amongst some researchers and land managers that removing weeds will automatically lead to positive biodiversity outcomes, with the more weed removed, the better the outcome. However, this is often not the case, particularly for long-established weed species whose dominance has created impoverished communities with little capacity for passive recovery. A common result may be wasted investment in weed control and, in the extreme, net negative impacts upon asset values. We present a conceptual model for the management of weed-impacted assets, plus guidance for its application, with a view to improving asset recovery practice. Weed removal should be calibrated by asset recovery, which may mean not seeking to completely remove a weed at a given spatial scale. Our model focusses on weed removal that is enough to initiate asset recovery, but not more than is necessary to promote maximum expression of asset resilience, particularly in the context of secondary invasions. Optimal management efficiency will involve a proportional allocation of resources to control, monitoring and revegetation activities that is appropriate to the stage of asset recovery, as well as a willingness to revise a management goal if the original one cannot be achieved within existing constraints on resources.

Keywords
Biodiversity, natural asset recovery, resilience, resistance, secondary invasion, weed management

Introduction

Considerable attention has been paid to early intervention in the management of invasive alien plants, especially those whose potential impact upon the values of natural ecosystems is high (Panetta and Cacho 2014, Panetta 2015, Blood and James 2016, Wilson et al. 2017). Procedures for risk assessment and the planning, implementation and evaluation of incursion management strategies (e.g. eradication and containment) are well-established (Wilson et al. 2017). Conversely, after invasive plants become widely established, the focus of management necessarily switches to asset protection, with the aim of protecting the greatest ecological values of threatened communities (Downey et al. 2010, Shackelton et al. 2017). Where extirpation of an invader is not feasible, asset protection can be achieved proactively by maintaining its abundance below an impact threshold, defined as the abundance beyond which the asset in question (e.g. native plant community diversity) becomes increasingly degraded with further increases in invader abundance (Panetta and Gooden 2017). This approach relies upon the **resistance** (Nimmo et al. 2015) of natural ecosystems to invasion, i.e. their ability to maintain biodiversity in the presence of an invader at abundances below the impact threshold.

Elsewhere, a common goal of management is to improve biodiversity values of impacted communities that have become impoverished through the dominance of invasive plants over time (Reid et al. 2009, Kettenring and Adams 2011), although other management goals (such as restoration of native species cover or provision of ecosystem services) may be pursued, depending upon site history and landscape context (Gaertner et al. 2012). In such cases the approach to management is essentially reactive and highlights the importance of plant community **resilience** in restoration (i.e. the capacity for recovery once the invader has been removed). Two pervasive assumptions underpin weed management for the recovery of impacted biodiversity: (1) that the weed should be removed entirely from the focal area of interest (Reid et al. 2009); and (2) that the removal of the weed will enable recovery of the asset (e.g. Vosse et al. 2008). Some progress has been made regarding the second question, particularly in our understanding that weed removal technique may modulate asset recovery trajectories (Mason and French 2007, Flory and Clay 2009). However, the assumption persists that removing a weed entirely will yield the best outcome for biodiversity, and the retention of a weed at any level of abundance within the target control area over time is unacceptable.

In this paper, we show how a greater focus on the response of native biodiversity assets to control actions may improve management outcomes for long-established weed species. We develop a conceptual model that can be used to evaluate asset recovery in response to weed management where operational resources are scarce and the capacity for passive restoration is unknown. Our approach integrates three key sets of questions and considerations:

1. What is the potential for passive recovery of a given asset, and how much weed removal is necessary to achieve this potential? Here, we consider impact thresholds of weed removal on the regenerating asset and identify ceilings to passive recovery, after which active regeneration will be required to improve asset condition. The role of community resilience to invasion in determining the relative importance of passive and active asset recovery is highlighted.
2. What is the likely impact of the establishment of other invaders facilitated by management ("secondary invasion"; Pearson et al. 2016a, O'Loughlin and Green 2017, Nsikani et al. 2018) upon asset recovery, and under what circumstances might secondary invasions force a reconsideration of management goals? Here, we highlight that secondary invasions are among the most common and pervasive outcomes of weed management and, as such, need to be accounted for in any model that considers asset response to primary weed removal.
3. How should management effort be allocated over time between the main activities (weed control, monitoring and revegetation) associated with asset recovery? Here, we define three stages of asset recovery that differ according to the optimal relative allocation to various activities.

Answers to these questions will assist land managers to determine the most cost-effective means of achieving articulated management goals and provide a basis for goal modification if required. We focus on the case of a single invasive species but see no reason why our conclusions would differ qualitatively in the presence of multiple primary invaders. However, we acknowledge the current deficiency of empirical observations relevant to our model and thus offer it for the purposes of informing management practice and stimulating further research.

Why weed removal actions should be guided by asset recovery

A common theme in the control of invasive species is a failure by researchers and practitioners to take a whole-of-community approach to evaluating the efficacy of weed management in recovering target assets (Kopf et al. 2017). Managers frequently monitor target weed responses to management as a surrogate for native asset responses that remain unmeasured (Reid et al. 2009; Foster et al. 2019), and financial constraints often preclude monitoring beyond one or a few growing seasons (Kettenring and Adams 2011). Similarly, reviews and meta-analyses of invasive plant control research have found that while most studies measured the effect of management on the target invasive species, far fewer also assessed the response of native plant species (estimates range from 19% to 30%) (Reid et al. 2009, Kettenring and Adams 2011; for a recent example of native species response over the medium term see Ruwanza et al. 2018). Emphasis on the response of the target invasive species commonly finds expression in project operational milestones, for example area of weed infestation cleared, with an assumption that weed removal benefits resident biodiversity. Subsequently, there is very limited understanding of the spatial or temporal scales over which native communities recover post invasion, whether recovery progresses along a trajectory towards a desired native condition, or if the method optimal for weed removal is also optimal for asset recovery. Moreover, impacted communities are often fundamentally altered to the extent that the pre-invasion condition (as observed in non-invaded reference sites; Hudson et al. 2014, McDonald et al. 2016) may not be readily, or ever, restored.

It is surprising that the idea of a strong link between weed removal and asset recovery persists, given that examples of successful passive restoration of invaded communities are extremely rare (Pearson et al. 2016a, Prior et al. 2018). Passive restoration usually succeeds only when the native plant community has a high degree of natural resilience to invasion, the surrounding landscape provides high native propagule pressure, and disturbance is not severe (Holl and Aide 2011). Natural resilience is likely a function of the period over which a community has been invaded, in situ persistence of native species in the form of underground vegetative propagules or seed banks, and the potential for native species immigration post invasion. Optimising weed management for the restoration of invaded communities therefore hinges upon knowledge of recovery trajectories, responses to weed management extent and method, and identifying limitations to passive regeneration beyond which active revegetation will be required.

Numerous factors contribute to the difficulty of effective passive restoration. These include legacy effects of invasion (Yelenik et al. 2004, Corbin and D'Antonio 2012, Gioria and Pyšek 2016), secondary invasions (Kettenring and Adams 2011, Pearson et al. 2016a, González et al. 2017a), non-target effects of invader control (Mason and French 2007, Rinella et al. 2009, Skurski et al. 2013), and variation in the resilience of the community (Prior et al. 2018). Also to be considered are the positive effects that some invaders have, such as the nesting sites and protection afforded by invasive shrub species to birds, reptiles and small mammals in disturbed landscapes (D'Antonio and Meyerson 2002). Management of long-established invaders requires an ecosystems outcome approach that takes all of these factors into consideration (D'Antonio and Meyerson 2002; Kopf et al. 2017). Setting realistic and achievable management goals for restoration is essential (Hobbs 2007) and goals need to be considered in the dual context of asset condition and available resources. Proper monitoring of the asset recovery response to management actions should help managers to invest limited resources most efficiently and cost-effectively.

Important thresholds and ceilings in restoration responses to weed management

Currently, guidelines have been formulated for impact and action thresholds where plant invaders are managed **proactively**; i.e. before they have reached enough abundance to impact the target natural asset (Panetta and Gooden 2017). However, there appears to have been no consideration of thresholds where the **reactive** management goal is to improve the biodiversity status of communities that are already impacted by a widespread invader. Our model comprises three main points, which taken together comprise asset responses to weed removal (see details in Box 1 for detailed explanation of the model, and a glossary of key terms in Box 2):

Asset recovery threshold

The first is an **asset recovery threshold**, which denotes the minimum amount of a weed that must be removed to initiate passive asset recovery (e.g. near point **1** on curves **A**, **B** and **D**, and point **3** on curve **C**; Box 1). The position of this threshold (corresponding to the area of weed removed) will depend upon the spatial pattern of propagules of native species, occurring in situ or arriving via immigration post weed removal. High resilience may be conferred by a dense, species rich native seed bank, with seedling recruitment into the standing vegetation commencing after the target weed is removed.

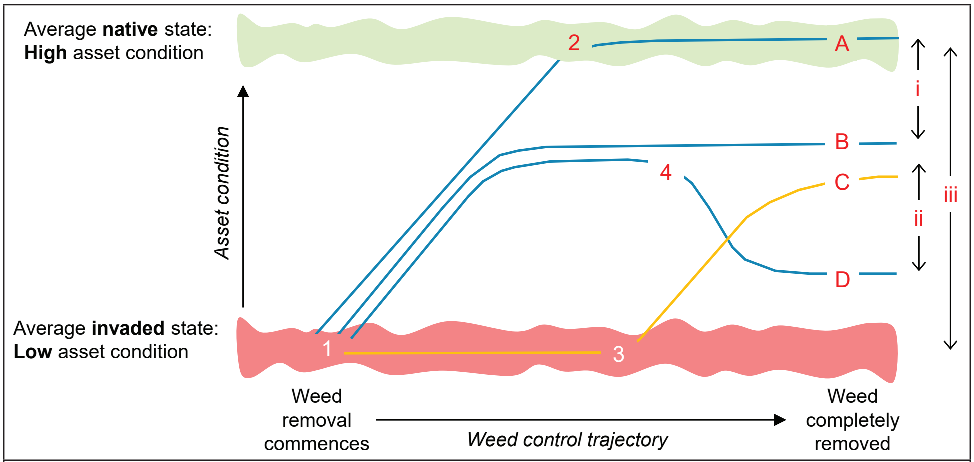
Asset recovery ceiling / weed removal ceiling

The second is an **asset recovery ceiling** or **weed removal ceiling**, beyond which asset condition will not passively improve with further investment in weed removal (point **2** on curve **A**, plus analogous inflection points on the other curves; Box 1). In the most favourable case, i.e. where community resilience is very high, the asset recovery ceiling will coincide with the most desirable uninvaded native state (point **2** on curve **A**; Box 1). Otherwise, a **recovery deficit** (interval **i**; Box 1) will occur, after which active intervention, such as planting seeds or nursery-grown seedlings of some native species, will be required to facilitate recovery to the desired native reference state. An asset recovery ceiling may be reached if certain plant species with short-lived seeds or short-distance dispersal mechanisms are eliminated by the invader and subsequently are unable to recolonise the site.

Management impact threshold

The third is a **management impact threshold** (point **4** on curve **D**; Box 1), which occurs when further weed removal, substantially beyond the asset recovery ceiling, would cause the asset to deteriorate, owing to unintended negative effects of control actions. Such effects may be direct, such as damage to native plant species due to herbicide drift, plus soil compaction, erosion and nutrient loss. They may also be indirect, for example by promoting secondary invaders that suppress native regeneration and may be more difficult to manage than the target weed species (Cox and Allen 2008, Ortega and Pearson 2010). Indirect negative impacts may arise as well through a loss of unforeseen beneficial effects of the targeted weed (e.g. the nesting sites and protection afforded by invasive shrub species to birds, reptiles and small mammals; D'Antonio

Box 1. Conceptual diagram of the model for the recovery of weed-impacted natural assets.



Axes

The *y*-axis represents a sliding scale of vegetation community condition between an average invaded state (red zone) and an average non-invaded, native reference state (green zone). The origin at point **1** represents a state 100% dissimilar to the non-invaded, native condition (e.g. a weed-dominated area that contains none or a different suite of native species to those found in non-invaded reference areas). It is assumed that a non-invaded, native state comprises higher asset condition than weed-dominated areas. Impact of invasion is measured as the magnitude of difference between the average non-invaded state (green zone) and the average invaded state (red zone), represented by interval **iii**.

The *x*-axis represents a sliding scale of weed removal. The curves **A**, **B**, **C** and **D** represent different patterns of vegetation community response to weed removal (i.e. *regeneration trajectories*). Vegetation community regeneration in response to weed removal will vary as a function of *resilience* to invasion, which is defined as the extent of recovery of the asset post invasion (Nimmo et al. 2015). Blue curves (**A**, **B** and **D**) represent communities with relatively high resilience to invasion, while the orange curve **C** represents a community with relatively low resilience (see description below). For simplicity, we have presented *resistance* to invasion (i.e. degree of community change in response to invasion, interval **iii**; Nimmo et al. 2015) as equal for all curves, hence why the level of impact is equivalent at point **1** for all curves at the maximum level of weed abundance before removal of the weed commences.

Regeneration trajectories

Curve **A** represents the most resilient community (the best-case scenario for managers), because the native vegetation begins to recover very soon after weed removal commences (i.e. the *asset recovery threshold* occurs close to point **1**), any amount of weed removal facilitates native vegetation recovery, and full recovery to the reference native state is achieved. An *asset recovery ceiling* occurs at point **2**, beyond which asset condition will not improve with any further investment in weed removal. This point can also be considered as a *weed removal ceiling*, the latter being causal and asset recovery its effect.

Curve **B** is similar initially to curve **A** in that removal of the weed initiates rapid, linear asset recovery near point **1**. However, for curve **B**, the community is relatively less resilient to invasion because the response trajectory does not reach the maximum level of the desired native reference state by the time the asset recovery ceiling is reached. This results in a *recovery deficit* (interval **i**) and represents the model space in which active intervention would be required to facilitate full community recovery (e.g. planting nursery-grown seedlings or seed addition).

Curve **C** represents a community with a much-reduced level of resilience, since regeneration of the asset only commences after a much greater proportion of the weed is removed from the invaded site (i.e. asset recovery threshold at point **3**).

Curve **D** represents a scenario where weed removal promotes asset recovery until a *weed removal ceiling* is reached, but thereafter inhibits asset recovery due to disturbance effects of the control technique being used to remove the weed. In this case point **4** represents a *management impact threshold* and interval **ii** represents the net effects of management that balances benefits of weed removal (maximum at point **4**) with negative effects of control action used to remove the weed.

Box 2. Glossary of key terminology.

Term	Definition
Asset recovery ceiling:	Maximum level of passive asset recovery in response to weed removal.
Asset recovery threshold:	Minimum level of weed removal required to initiate asset recovery.
Ecological resilience:	Magnitude of asset recovery in response to weed removal. Plant communities with low levels of resilience will experience limited recovery post weed removal and remain in a degraded state without active revegetation.
Ecological resistance:	Magnitude of change in asset condition in response to weed invasion, usually calculated as the difference in asset condition between weed-dominated and native reference sites.
Management impact threshold:	Level of weed removal beyond which management inhibits asset recovery.
Recovery deficit:	Difference between the asset recovery ceiling and the level of asset condition in native (i.e. non-invaded) reference sites. Recovery deficits can be bridged using active regeneration actions (e.g. by reintroducing plants with short-distance dispersal mechanisms as seed or nursery-grown seedlings).
Regeneration trajectory:	Pattern of change in asset condition through time in response to weed management.
Weed removal ceiling:	Maximum level of weed removal at which maximum asset condition (i.e. asset recovery ceiling) is reached.

and Meyerson 2002). In extreme cases, extending weed removal beyond the asset recovery ceiling could result in the condition of the native asset regressing to a state that is more degraded than its initial condition. In more moderate cases, reducing weed cover beyond the management impact threshold would not only reverse gains made to asset condition (note the decline in asset condition beyond point **4** on curve **D**), but also waste money, time and resources that could otherwise be allocated to restoration and maintenance at the site in the future, or weed control elsewhere. It follows that this threshold is one that practitioners should take precautions not to breach.

Secondary invasion: a fundamental impediment to asset recovery

As was foreshadowed in the Introduction, the responses of other invaders to primary weed removal will often play a critical role in determining the outcome of an asset recovery effort. In long-invaded sites, the seed bank is often dominated by non-native species (O'Loughlin et al. 2014; Gioria and Pyšek 2016) and removal of the targeted weed will likely give rise to secondary invasions by one or more species. This is a prime reason why restoration efforts fail: in a meta-analysis of 60 cases from 38 studies Pearson et al. (2016a) found that control efforts markedly reduced primary invader abundance, but the consequence of primary invader control was usually secondary invasion, not the recovery of native species. The increase in secondary invaders was associated with a mean effect size twice that for native plants, which increased only weakly. Pearson et al. (2016a) concluded that primary invader suppression was the key factor in the release of secondary invaders and argued that management strategies are required that anticipate and suppress secondary invaders as part of site restoration.

Sometimes the abundance of secondary invaders will decrease over time post control, even in the absence of further management intervention. In some mesic environments, for example, secondary invasion comprises shade intolerant, short-lived ephemerals that are displaced as native vegetation develops. Gooden et al. (2009) found that after the removal of the non-native shrub *Lantana camara* in wet sclerophyll forest on the southeast coast of Australia, secondary invaders comprised transient annual and biennial herbs that were unlikely to impact on regenerating native species. Similarly, there was only a temporary spike in exotic species richness in a New Zealand wetland following the aerial application of glyphosate to a *Salix cinerea* infestation (Burge et al. 2017). In other situations, however, the potential impact of secondary invaders may be greater than that of the targeted weed (Dickens et al. 2016, Pearson et al. 2016b), or their management more difficult. The second possibility would appear to be more important in the context of plant community recovery. Cox and Allen (2008) found that when non-native annual grasses were controlled by a grass-selective herbicide in southern California coastal sage scrub, non-native forbs, especially species of *Erodium*, increased in cover. The effects of *Erodium* on the emergence and establishment of native species are variable (see references in Cox and Allen (2008)) but the further option of selective removal of these non-native forbs with herbicides is clearly not available. In bunchgrass communities of western Montana, Ortega and Pearson (2010) found that control of the invasive forb *Centaurea stoebe* with picloram was effective on the targeted species, but afterwards dominance shifted to the non-native grass *Bromus tectorum*, at the expense of native grasses. Thus, application of grass-selective and broadleaf-selective herbicides in these two cases led to difficult-to-manage secondary invasions by the complementary life form.

Using this model in practice: monitoring, secondary invasions and recovery deficits

Where monitoring and evaluation of weed control programs is undertaken, this commonly occurs at the completion of the program (FD Panetta, personal observations). However, monitoring and evaluation during programs is far more important, potentially providing evidence of significant off-target effects on the asset, or other reasons to modify management actions to achieve stated goals (Hulme 2006, Field et al. 2007). Timely delivery of information of this sort can also help land managers to decide whether management goals as originally articulated are achievable. In the present case, only through appropriate monitoring can weed control actions be informed by asset recovery trajectories.

Determining the asset recovery threshold

Although multiple thresholds and ceilings are identified in the model, its practical implications are relatively straightforward. If the weed-impacted asset has any degree of natural resilience to invasion, its recovery threshold (i.e. the minimum level of weed removal necessary to initiate asset recovery) should be exceeded at some point early on during the weed removal process, so identification of this threshold will usually not be critical. However, practitioners need to have the capacity for substantial weed removal in the event that the recovery threshold is not reached until a large proportion of the weed population has been removed.

What is critically important is the weed removal ceiling that is associated with the asset recovery ceiling. If weed removal ceilings are properly identified during asset recovery operations, there will be less chance of: (a) wasting management effort through superfluous weed control, or (b) exceeding the management impact threshold, at which point the net effects of weed control would become negative (see point **4** on curve **D**; Box 1). Furthermore, management effectiveness will be improved when managers can redirect resources from unnecessary weed removal to active restoration efforts to bridge the recovery deficit. Several steps need to be taken prior to attempting asset recovery. Based upon an assessment of the asset's degraded condition prior to weed removal, the management goal must be clearly defined, i.e. what is the desired degree of asset improvement? Next, a monitoring schedule needs to be established, taking into consideration the variables that will be measured as a surrogate for asset recovery in response to weed removal, plus when monitoring will be undertaken (see below).

Identifying the weed removal ceiling

A logical approach for identifying the weed removal ceiling would be to remove the targeted weed incrementally, as illustrated in Figure 1. Incremental removal would contribute to suppression of the target weed and provide a means of assessing asset resilience (i.e. the size of the recovery deficit; see interval **i** in Box 1), as well as the potential for secondary invasion. The initial area subjected to weed removal, and the pattern of weed removal within the area, should be determined with a view of gaining an estimate of passive recovery potential. Contrary to the conventional practice of some contractors who expend a major proportion of the planned control effort to a single weed removal operation, a more suitable approach would involve weed removal operations that are staggered through time and space. Such a staged approach is usually undertaken by volunteer restoration practitioners, yet their valuable on-ground experiences are rarely integrated into broad weed management strategies at higher governance levels (O'Meara and Darcovich 2015, Peters et al. 2015, Pagès et al. 2019). Enough time should be allowed for the recruitment of native species after each operation. The time required for a proper assessment of the passive recovery response will vary according to environmental factors, especially rainfall incidence (Ogden and Rejmánek 2005, Cox and Allen 2008, González et al. 2017b) and the occurrence of critical germination cues, such as fire (Lindenmayer et al. 2015).

Evidence of asset resilience should be interpreted in the context of observations on the presence and nature of secondary invasions, especially the ease with which such

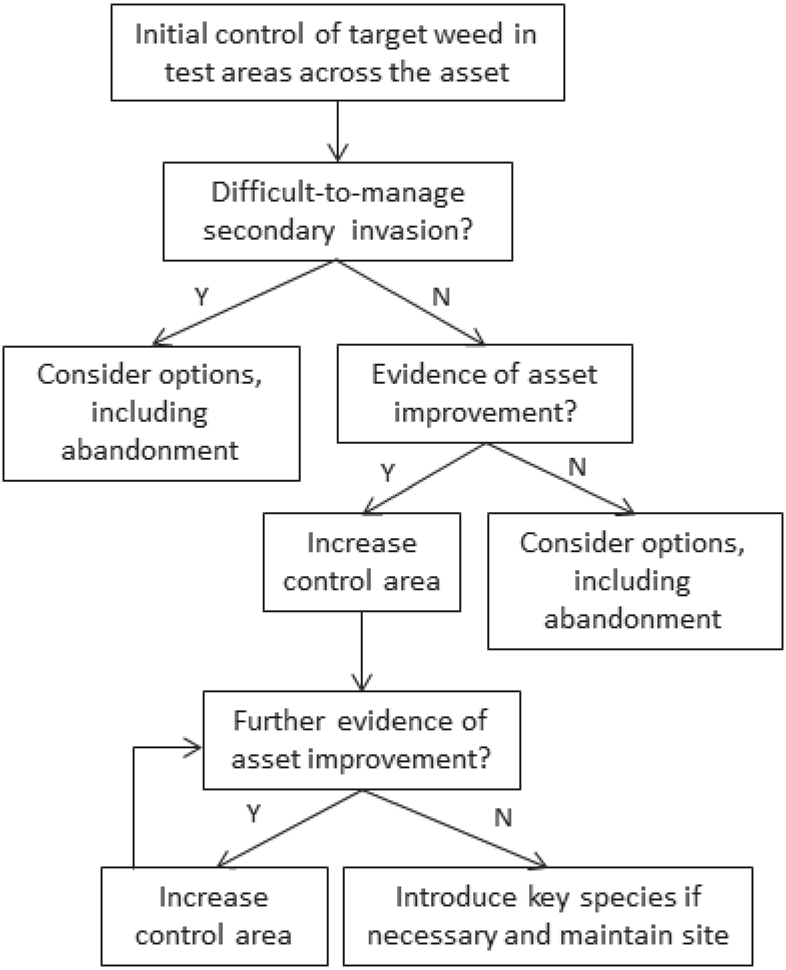


Figure 1. Decision tree for determining primary weed removal ceilings. Evidence of asset improvement includes increases in native species richness and evenness, and increased similarity of community structure to that found in reference sites. If the asset response following initial weed removal includes problematic secondary invasions, reconsideration of the initial management goal may be appropriate.

invaders could be managed while maintaining the restoration objectives for the asset (Figure 1). As Pearson et al. (2016b, p. 16) state, "In situations when the likelihood of promoting problematic secondary invaders is high and mitigation strategies for such invaders are lacking, the no action management alternative may be advisable." Implementation of highly targeted control (e.g. treating individual plants) will reduce the size of management-induced disturbance, hence reducing the opportunity for secondary invasion (Pearson et al. 2016a).

The extent of active revegetation required will depend upon the level of community resilience to invasion. With high resilience, perhaps only a few key species (with emphasis on missing or poorly represented functional groups) will need to be introduced, either as seed or vegetative stock, for the asset to gain an acceptable level of similarity to non-invaded reference sites. Where low resilience is evident, the amount of active revegetation that occurs will depend upon the resources available for management. The potential for increasing biotic resistance to secondary invasion should also be considered when selecting species for reintroduction (Schuster et al. 2018). In many cases the availability of volunteer participation over long periods will be crucial to asset recovery (O'Meara and Darcovich 2015, Peters et al. 2015, McDonald et al. 2016, Pagès et al. 2019). Here, the costs of materials (e.g. native species seed and tube stock) will generally be small relative to the value of the voluntary labour component. In terms of total effort and investment, management options thus range from abandonment of asset recovery altogether to reintroduction of a number of native plant species (Figure 2).

Dealing with asset recovery deficits

Asset recovery activities can be partitioned between invader control, active revegetation and monitoring. In a successful restoration program, the relative allocation of total effort to these activities can be expected to change over time (Figure 2). While more than one activity will always be required in any one stage, the predominant activity will be weed control, revegetation and monitoring in Stages 1, 2 and 3 respectively. Irrespective of the management goal, Stages 1 and 3 in this scheme are potentially the least variable, with the predominant activities in Stages 1 and 3 being weed removal and monitoring, respectively. The nature of Stage 2, however, may vary significantly between assets, depending upon the size of the recovery deficit (see interval **i** in Box 1), which will determine the required allocation to revegetation (Figure 2). Land managers may decide that the recovery deficit is too large to address (but see O'Meara and Darcovich 2015, Peters et al. 2015, and Pagès et al. 2019 for cases of monitoring and asset recovery via the contributions made by volunteers over long periods), or the existence of legacy invader effects may render restoration to reference native conditions unfeasible—at least until such effects have been ameliorated. Where the recovery deficit is high and secondary invasion is a significant issue, asset recovery can effectively stall in Stage 1 (Figure 2), requiring a reconsideration of management goals.

Where the asset's recovery deficit is moderate, secondary invasion is minimal or easily managed and there are enough resources for revegetation, the duration of Stage 2 will depend upon the rates of establishment, survival and growth of the native species utilised in revegetation. In the case of a low recovery deficit (see curve **A** in Box 1 and Figure 2), however, Stage 2 may be brief or even absent.

Unless the goal of improving the biodiversity of assets is abandoned, an indefinite control effort will be required to prevent resurgence of the primary invader or emergence of a new one (Stage 3). In addition to factors such as the level of participation by local volunteers, the frequency of monitoring during Stage 3 will depend upon invader population dynamics, especially the time to reproduction (see Panetta 2015), the rate of biomass accumulation and levels of immigration from the surrounding landscape.

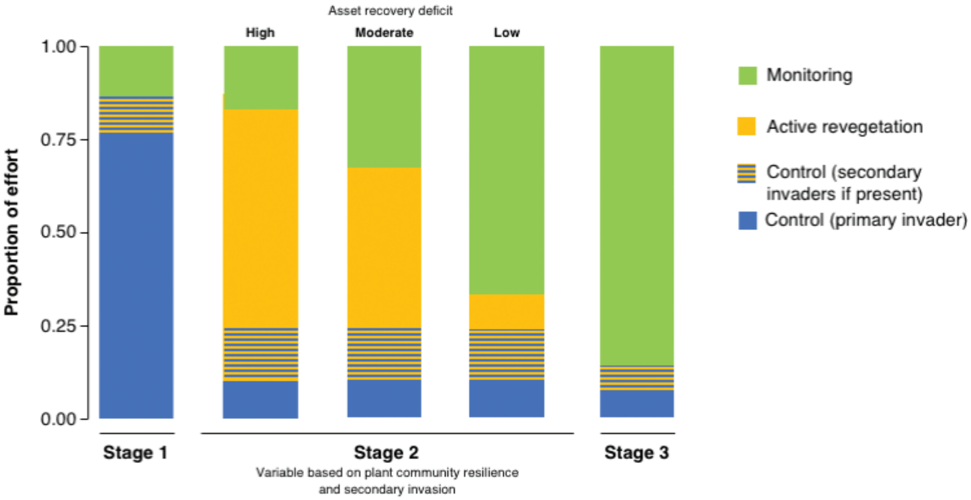


Figure 2. The proportional allocation of effort to various activities will change through time during asset recovery programs. The proportions of effort allocated to activities in Stage 2 with be dependent on identifying the asset recovery threshold and asset recovery ceiling specific to each program (see Box 1). A protracted requirement for the control of primary or secondary invasions indicates a stalled program that is unlikely to transition to Stage 2, demonstrating a need to reconsider the initial management goal. Note that values portrayed are for illustrative purposes only.

Balancing the costs and benefits of asset recovery activities

Incomplete evaluation of costs and benefits associated with invasive species management actions poses a key barrier to successful invasive species control (Kettenring and Adams 2011). Costs and benefits are most frequently considered in terms of methodology (i.e. the relative efficacy of control action in killing or preventing the reproduction or spread of invasive species) and the strategic allocation of resources (i.e. when, where and how much control should be applied to manage invasions) (Epanchin-Niell and Hastings 2010). Since repeated control methods are often required to manage invasive species effectively, the costs and benefits of specific control methods must be considered when implementing control programs (Meloche and Murphy 2006). Generally more emphasis has been placed on the costs of invasive species control than on the resultant benefits (e.g. increased native species richness and improved vegetation structure), as evidenced by the plethora of weed control studies or operations where evaluation has concerned target effects to the exclusion of asset recovery response (Reid et al. 2009, Kettenring and Adams 2011).

Only when the benefits resulting from targeted control activities are quantified will it be possible to make proper decisions regarding the management of weed-impacted assets. If there is no evidence of positive asset response after weed removal operations (see Figure 1), the costs of the operations will unquestionably outweigh the benefits. Costs will also exceed benefits should the total amount of weed removal breach the management impact threshold (see point **4** on curve **D** in Box 1), but if weed removal is incremental and monitoring is undertaken this outcome can be avoided. Positive impacts of the targeted invader (Kumschick et al. 2012, Blackburn et al. 2014) also need to be included in the calculus. With longstanding weed invasions, the primary task of the land manager is to ensure that the costs of weed control are equal to or less that the derived biodiversity benefits; timely assessment of asset recovery status is therefore an essential part of this.

It would be pointless to invest in 100% weed removal if this provides no additional benefit to biodiversity when compared to removal to a lower level (e.g. 50% of initial abundance) and maintaining this level over time, especially when negative impacts of weed removal on the target asset are evident. However, there may be circumstances where total weed removal is appropriate. Here, subsequent asset recovery may be highly dependent on the quality and sensitivity of the weed-removal technique employed, which either promote or hinder seed bank initiation of community regeneration (Vosse et al. 2008). For example, the regeneration of fynbos vegetation in the Western Cape Province of South Africa was poorest when the woody invaders *Pinus radiata* and *Hakea sericea* were felled and then burned, owing to the excessive heat generated by large amounts of dead fuel (Holmes et al. 2000). In Colorado, mechanical clearing of *Tamarix* species using a Hydro-Ax (a piece of heavy equipment designed for mowing woody vegetation, leaving all debris on-site), did not cause a resurgence of *Bromus tectorum* and other invasive species, most likely due to the mulching effect (Sher et al. 2008). These two examples demonstrate that methods of broadscale weed removal may have consequences for both native species recruitment and the level of secondary invasion. Accumulated costs that greatly exceed apparent benefits signal a need for a change in the management goal; the options under consideration must include disinvestment.

Concluding remarks

The prevailing perspective on the management of invasive plants in natural assets is one in which the explicit focus is on implementation of invader control to obtain biodiversity benefits via an implicit focus on asset recovery. It is understandable, therefore, that the assumption took hold that invader removal would automatically lead to ecological improvement. With our model and its application we are proposing a subtle, but important, change in perspective, whereby the explicit focus is on recovery of the asset, based on direct monitoring of its response to invader control.

Prevalent funding models pose a major hindrance to the efficacy and cost efficiency of attempts to effect plant community recovery. Funding for invasive species control is often allocated in parcels that are unlinked to restoration or conservation funding (Prior et al. 2018). This helps to explain why population-level management, with the assumption that weed removal will automatically lead to biodiversity gains, has been a common approach to managing invaded natural ecosystems (Prior et al. 2018). Our proposed staged approach to asset recovery, one that combines assessments of both recovery deficit and the intrinsic limitations to recovery, runs counter to contractual arrangements that require relatively rapid expenditure of funds on primary weed control, presenting a potentially serious impediment to its implementation. This underlines the need for managers to work closely with volunteer restoration practitioners in designing and implementing weed removal programs. Moreover, the availability of accurate measures of the benefits of alien clearing may be key to identifying and accessing additional avenues for funding.

Realistic asset recovery goals can be formulated only by taking multiple factors into account, including legacy effects, asset recovery deficits, manageability of secondary invasions and, perhaps most critically, resource availability. The primary operational objectives should be to quantify the recovery deficit and the potential limits to recovery posed by secondary invasion. Land managers must always be prepared to revise management goals if timely monitoring and evaluation point towards a lower degree of asset recovery than was originally envisaged. Biodiversity benefits may still be obtained via a lesser asset recovery, but failing this, managers must be prepared to disinvest altogether.

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RESEARCH ARTICLE

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Steatoda nobilis, a false widow on the rise: a synthesis of past and current distribution trends

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Abstract

The Noble False Widow, *Steatoda nobilis* (Thorell, 1875) (Araneae, Theridiidae), is, due to its relatively large size and potential medical importance, one of the most notable invasive spider species worldwide. Probably originating from the Canary Islands and Madeira, the species is well established in Western Europe and large parts of the Mediterranean area and has spread recently into California and South America, while Central European populations were not known until 2011.

We report on long-time observations that reveal that at least two flourishing populations in Germany (Cologne) have been present for over five years, while in Ecuador one population has been observed between 2014 and 2018 and several other records were made in other parts of the country. Data obtained from the British Spider Recording Scheme demonstrate that the species moved significantly northwards since the report of the first populations in the very South of England, after several decades of relative stasis. The sudden northward expansion highly correlates with a massive rise in press coverage of the species.

In the Americas, *S. nobilis* is currently known from four countries (USA, Chile, Ecuador, Colombia), and available DNA barcoding data obtained for specimens from this area suggest that multiple introductions occurred within each country. Using ecological niche modeling, we identified suitable climate regions for the species and discuss possible reasons for its current spread. We propose that seaside cities and villages with a temperate oceanic or Mediterranean climate are especially favourable potential habitats for *S. nobilis* and will face the highest colonization pressure in the future, while tropical upland regions with temperate climates are also vulnerable to invasion by *S. nobilis*.

Keywords
alien species, araneism, biological invasion, citizen science, spider, steatodism

Introduction

With currently over 47 000 described species, spiders (Araneae) represent a hyperdiverse and extraordinarily variable arthropod group inhabiting nearly every terrestrial habitat on the globe (Mammola et al. 2017; World Spider Catalog 2018). Numerous species are successful alien colonizers (e.g., Kobelt and Nentwig 2008; Nentwig 2015) and spread, assisted by human activities, into natural and human-influenced habitats all around the world (e.g., Levi 1967; Pugh 2004; Nentwig 2015).

Members of the spider family Theridiidae, also known as tangle-web or cobweb spiders, are among the most successful alien spider species (Levi 1967; Nentwig 2015). Many alien theridiids live in and around human settlements (Kobelt and Nentwig 2008), and some particular species, e.g., *Nesticodes rufipes* (Lucas, 1846), *Steatoda grossa* (C.L. Koch, 1838), and *Parasteatoda tepidariorum* (C.L. Koch, 1841), are common house spiders in many parts of the world (Levi 1967). In some cases, alien theridiids seem to be able to replace native spider species of conservation importance (Hann 1990) or compete successfully against other native house spiders (Nyffeler et al. 1986), as well as threatening other arthropods through predation (Bryan et al. 2015) or even hybridizing with native species (Vink et al. 2008). Also, some theridiids of medical importance, e.g., black widow spiders of the genus *Latrodectus*, show a considerable range expansion due to human assistance. The infamous Australian redback spider, *Latrodectus hasselti* Thorell, 1870, successfully invaded New Zealand and Japan (Nihei et al. 2003; Vink et al. 2011), and the brown widow, *Latrodectus geometricus* C.L. Koch, 1841, a less dangerous relative (Müller 1993), was introduced into several countries and established on at least four continents (Vincent et al. 2008; World Spider Catalog 2018).

Steatoda nobilis (Thorell, 1875), one of the largest theridiids and sometimes called false widow or noble false widow, is often mistaken for a *Latrodectus* species. This species is able to inflict a painful bite often accompanied by swelling, erythema, and pruritus, but normally no systemic effects occur after a bite incident (Dunbar et al. 2017). The envenomation is partially comparable to that by *Steatoda grossa* (Isbister & Gray, 2003). Nevertheless, alleged *S. nobilis* bites are a stock feature especially of the British popular press (e.g., Christodoulou 2018), and reports of *S. nobilis* infestations have regularly resulted in public disruption (e.g., Associated Press 2015; Siddique 2018).

Probably native to the Canary Islands and Madeira and first described from the latter (Thorell 1875), *S. nobilis* dramatically expanded its range in the last 100 years. The main invasion pathways for several alien spider species are relatively well known (potted plants, fruits, containers, packing material, and feeder cricket boxes) (Nentwig 2015), and for *S. nobilis*, it was often suspected that current populations in Great Britain and Ireland are descendants of specimens imported to Europe with bananas and other fruit from the Canary Islands (e.g., Roberts 1995; Kulczycki et al. 2012).

Although alien populations of *S. nobilis* are known today from England, Ireland, several parts of the Mediterranean area, California, and Chile (Snazell and Jones 1993; Vetter et al. 2015; Taucare-Ríos et al. 2016; Dugon et al. 2017), no established population has ever been reported from Germany or other Central European countries. In the Mediterranean area and, e.g., California, nearly all records of *S. nobilis* are located in low altitudes, often near the coast (Kulczycki et al. 2012; Vetter et al. 2015; Morano et al. 2018), while the species is known to occur in natural habitats at altitudes up to 3200 m in their native range on Tenerife (Schmidt 1968).

Recently, we became aware of two established populations in Cologne, Germany, and collected *S. nobilis* in domestic settings in urban areas in the uplands of Ecuador. Both of these records are in accordance with the current spread of *S. nobilis* into new and sometimes unexpected habitats and areas all around the world. To further explore the distribution of *S. nobilis*, we collated all known distribution data together with a large citizen science dataset from Great Britain and performed several analyses of the current distribution trends on a global and local scale, including a global species distribution model. The aim of the work is to present a brief review of the invasion history of *S. nobilis*, describe current and potential future problems with this invasive species, and to identify regions which are suitable for future invasion.

Material and methods

Field surveys

Spiders were observed and collected by CW during several surveys at two localities in Cologne, Germany, in 2011–2017, with at least one survey per year. In Ecuador, several specimens were collected unsystematically by NR during a field trip in 2014. Collected specimens were identified using Snazell and Jones (1993) and the key in Nentwig et al. (2018). A male and a female found in October 2011 at locality 1 in Cologne (Tab. 1) were deposited in the collection of the State Museum of Natural History, Karlsruhe (**SMNK**). Two males and two females from Ambato were deposited at the Museo de Zoología (**QCAZ**) in Quito, Ecuador. Further material from Stuttgart, Germany, was also deposited at the SMNK.

Data review and analysis

To assess the global and local distribution as well as the invasion history of *Steatoda nobilis*, taxonomic and biogeographic literature was surveyed (Suppl. material 1: Table A1). Additional data were obtained from the British Spider Recording Scheme of

Table 1. Records of *Steatoda nobilis* in Germany.

Federal state	City	Location	Habitat	First collection	Status	Literature
BW	Stuttgart	48.8268N, 9.1677E	Wall of house	19.X.2018	?	Own data
NRW	Cologne	50.9657N, 6.8690E	Garden centre	10.X.2011	Established	Own data
NRW	Cologne	51.0139N, 6.9139E	Garden centre	XI.2011	Established	Own data
Berlin	Berlin	Not specified	Flower wholesale trade	2012–2013	Single observation	Reiser (2013)
BB	Not specified	Not specified	Garden centre	2012–2013	Single observation	Reiser (2013)
BW	Balingen	Not specified	Garden centre	2012–2013	Single observation	Reiser (2013)
Hamburg	Hamburg	Harbour area	Harbour buildings	Around 1954	Imports	Schmidt (1954, 1956)

the British Arachnological Society (British Arachnological Society 2018). We also performed a full-text search of the Lexis-Nexis database for all articles in UK newspapers mentioning "*Steatoda nobilis*". Subsequent analyses were done using the statistical software R 3.3.1 (R Development Core Team 2016). We used Spearman's rank correlation to analyse the relationship between number of records and number of press articles per year mentioning *S. nobilis.* To quantify the expansion of the species in Britain, the northerly distance of every grid record from Torquay, the site of the first and southernmost British *S. nobilis* record (Pickard-Cambridge 1879), was calculated using the function "distVincentyEllipsoid" in the R-package "geosphere" (Hijmans et al. 2017).

To further explore the global distribution of *S. nobilis* and its climatic drivers we performed additional analyses. We ran a principal component analysis (PCA) of bioclimatic variables (CHELSA climate data; Karger et al. 2017) to compare the native and invasive conditions in the environmental space. Based on these results we built a global species distribution model (SDM) using Maxent 3.4.1 (Phillips et al. 2017), a machine learning presence–background SDM which is widely used in macroecology (Merow et al. 2013). Detailed information about the PCA and the complete SDM analysis is provided in the Suppl. material 1. To test the predictions made by the climatic suitability model, a field survey was conducted in the city area of Granville, Normandy (France). The collected material was also deposited at the SMNK.

DNA barcoding data were obtained from the public section of the Barcode of Life Data System version 4 (BOLD), http://www.boldsystems.org/ (last accessed 1 May 2018). Specimens that were not determined to species in the database were identified on the basis of geographical and genetic proximity to identified specimens, as well as habitat information and habitus photographs available in BOLD.

Review and results

Global invasion history of *S. nobilis*

Steatoda nobilis was first described based on specimens from Madeira by Thorell (1875). Around the same time, a juvenile specimen of this species was collected by

Hamlet Clark in the neighbourhood of Torquay, England (Pickard-Cambridge 1879, as *Steatoda clarkii*). This record is often mentioned as the first record in Great Britain (e.g., Snazell and Jones 1993). However, this early record is slightly dubious, as in 1879 the Rev. Clark had already been dead for 12 years, and his lasting fame as a naturalist is based on his yachting trip (with John Gray) to Spain, Algeria, Brazil, and the Cape Verde Islands, during which he collected spiders for John Blackwall and, importantly, also made a brief onshore visit in Funchal, Madeira (in Tenerife, where the company also passed by, this was impossible due to quarantine restrictions) (Clark 1867). There is, thus, a distinct possibility of a mislabelling of the specimen, especially as Pickard-Cambridge explains that it was obtained together with many other spiders and had been "accidentally mislaid until very lately". Jackson (1907) reported on a specimen found on a cliff in Southern England "far from any house" and implied that an established population in England might exist. One of the earliest mentions of *S. nobilis* from the Canary Islands is found in Pickard-Cambridge (1908), who described specimens imported with Canarian bananas. Most probably *S. nobilis* was very regularly transported together with fruits from the Canary Islands to Great Britain, ever since Elder Dempster and Alfred Fyffe began the large-scale import of bananas and other fruits in the 1890s (Pickard-Cambridge 1908; Striffler and Moberg 2003).

In Germany, the species was one of the most frequently imported spiders with Canarian fruit arriving in the harbour of Hamburg at some point (Schmidt 1954, 1956), but there is no evidence *S. nobilis* ever became established in the Hamburg area. No further German specimens are deposited in the collection of the Zoological Museum of Hamburg, but three samples of imported specimens determined by Schmidt were transferred in 1988 to the collection of the Senckenberg Museum, Frankfurt (accession no. SMF 37425-115, 37426-115, and 37437-115; R. Klesser pers. comm.).

Although native to Madeira and the Canary Islands, *S. nobilis* was probably introduced on the Azores in the 20th century (Schmidt 1990). In the Mediterranean, it seems possible that populations of *S. nobilis* existed already before 1980: Pickard-Cambridge (1899) examined material from "Madeira and Spain" (the latter, however, could refer just to the Canary Islands), and Denis (1957) mentioned a juvenile specimen found in the Spanish Sierra Nevada determined as *Steatoda* sp. (as *Teutana*), which resembles *S. nobilis*. Additional records by Melic (1994), Vanuytven et al. (1994), and Snazell and Jones (1993) showed that *S. nobilis* was certainly already established at various places on the Iberian Peninsula in the early 1990s. A recent record of *S. nobilis* from the Normandy (Tab. 3) collected by TB, based on the predictions of the distribution model described below, represents the first clearly identified population from the French Atlantic Coast known to us (e.g., Emerit and Ledoux 2013; Courtial and Pétillon 2014).

The first record for Ireland was published by Nolan (1999), supporting the idea of a late establishment of *S. nobilis* in Western Europe. Today, the species is widespread on the island and common in many urban areas (Dugon et al. 2017).

In the Americas, the populations in California have to be established only recently. Vetter et al. (2015) pointed out that only very recently pictures and records of *S. nobilis* have been sent to an address originally used for records of *Latrodectus geometricus*, another alien theridiid which has settled in California. In Chile, records from four cities are currently known. Besides the records from Concepción and Temuco (Taucare-Ríos et al. 2016), observations of *S. nobilis* in Valdivia and Pucón are mentioned in Ceryngier et al. (2018). Concepción and Valdivia, both cities with large harbour areas, are separated by a distance of over 300 km. These records show that *S. nobilis* is widespread in Chile.

Steatoda nobilis has also been reported from Morocco (Denis 1962; Ledoux and Raphael 1998; Emerit and Ledoux 2013), which would be the only record for mainland Africa. However, Denis never formally published the numerous Moroccan records collected by Gattefossé (Emerit and Ledoux 2013). Additionally, the type locality of the closely related *Steatoda maura* (Simon, 1909) is located in Morocco (Simon 1909; Levy and Amitai 1982), and this species is mentioned as a possible synonym by Ledoux and Raphael (1998), without, however, giving arguments supporting this view. This synonymy had already been suggested by Wiehle (1934), without examining type material, but was convincingly refuted when Levy and Amitai (1982) examined and illustrated the types of *S. maura*. While this species is without doubt distinct, it is noticeably close to *S. nobilis* in terms of its genital morphology and overall habitus. Indeed, at the barcode level, the sequenced specimens most similar to *S. nobilis* are from BOLD:AAG5682 (distance 9.88%, reciprocal nearest neighbours in BOLD), three large adult females collected in Antalya and İzmir, Turkey. The photos provided by BOLD, together with the locality, indicate that these belong to *S. maura* (which has been reported from İzmir before; as *Lithyphantes gerhardti* from "Smyrna"; Wiehle (1934)). Therefore, material of *S. nobilis* from Morocco should be examined and compared to *S. maura*, but it still seems possible that *S. nobilis* is established in coastal cities of Morocco, as some other records originate from the very south of Spain (Morano et al. 2018). Incidentally, the relatively close genetic relation to the supposed *S. maura* also supports an origin of *S. nobilis* in the Macaronesian/North African region and not elsewhere (e.g., in the Americas). Interestingly, *S. maura* is not listed on current checklists of Turkey (e.g., Demir and Seyyar 2017). Based on the description of the only *S. nobilis* specimen by Türkeş and Mergen (2005, again listed by Türkeş and Mergen 2007) it is possible that these authors confused *S. maura* with *S. nobilis*, as the mentioned body length (6 mm) would be atypical for an adult specimen of *S. nobilis*. Additionally, the arachnological literature (Roberts 1995; Heimer and Nentwig 1991) used for identification by these authors does contain *S. nobilis*, but not *S. maura*. Another record of *S. nobilis* from India (Raiz Tabasum et al. 2018: 43) is based on a definite misidentification, like most of the species presented there (the associated image shows an unidentifiable theridiid, but certainly not a species of *Steatoda*).

First Central European populations of *S. nobilis*

Steatoda nobilis was collected and observed from autumn 2011 to October 2017 at two garden centres in Cologne, Germany, which are separated by a distance of about 7 km. The first specimen was sighted indoors on 10 October 2011 at a large garden centre located in the west of Cologne (Table 1). Subsequently, six adults and juveniles were collected the same day, but several more webs with the typical tubular retreat were located and indicated a high population density. A few weeks later (November 2011), the species was discovered indoors at the second locality (Table 1). On every visit over the subsequent years, several specimens and/or intact webs could be observed. At both localities, the species inhabited the gaps of the window profiles made of aluminium. Juveniles have been frequently observed on potted plants available for purchase. Since about 2014, the species seems to have colonized the outside walls of buildings at locality 1 as well as bushes in the parking lot. At locality 2, the species can be found on shelves in the outdoor area, as well as on an outdoor wall with common ivy. However, it is not known if these are permanent settlements, and the species possibly vanishes from adjacent outdoor habitats in cold winters. Additional singleton specimens were observed in garden centres in Balingen, Berlin and Brandenburg (Reiser 2013). We also searched for *S. nobilis* in about 10 other garden centres in Cologne and adjacent areas. No other specimens could be found. Although individual specimens of *S. nobilis* have been repeatedly found in Germany, the records from Cologne are the first evidence for established populations in Central Europe. Recently, on 19 October 2018, an adult male *S. nobilis* was collected on a wall near a garden centre in Stuttgart, Germany (Table 1, Fig. 5B).

First records of *S. nobilis* for Ecuador

We found *S. nobilis* in 2014 at several locations in the uplands of Ecuador (Table 2), at an average altitude of about 2800 m a.s.l. All records come from man-made structures in urban and rural habitats, mostly on walls along streets and on house walls, a similar microhabitat to that preferred by other invasive outdoor populations of *S. nobilis*, but in a rather different macrohabitat (Fig. 1). Adults of both sexes as well as juveniles were observed. The highest number of specimens could be found on a wall in Ambato-Montalvo. We revisited this population in February 2018, when several specimens of both sexes could be observed and collected again, which indicates an established population. In the population in Ambato, several females with egg sacs were observed.

Literature survey of the habitat preferences of *S. nobilis*

Together with these new populations, confirmed alien populations of *S. nobilis* are currently known from over 10 countries on four continents (Table 3, Suppl. material 1: Fig. A7). This allows a more detailed assessment of the habitat requirements of the species, which determine its invasive potential.

In Great Britain and Ireland, *S. nobilis* is very abundant in and around houses, and can be found on typical urban structures, e.g., in houses and on outside walls, concrete fences or hedges (Dugon et al. 2017; Snazell and Jones 1993). Dugon et al.

Table 2. Records of *Steatoda nobilis* in Ecuador.

Province	Location	Coordinates	Habitat	Date	Specimens	Leg.
Tungurahua	Ambato-Montalvo	−1.3266, −78.6257	Brick wall	10.X.2014	1 ♀	N. Reiser
Tungurahua	Ambato-Montalvo	−1.3256, −78.6270	Wall	17.X.2014	1 ♀	N. Reiser
Pichincha	Quito	−0.1857, −78.4781	Wall	07.XI.2014	1 juv.	N. Reiser
Tungurahua	Ambato-Montalvo	−1.3256, −78.6269	Wall	08.XI.2014	5 ♀, 2 ♂, juv.	N. Reiser
Pichincha	Quito	−0.1860, −78.4795	On house wall	10.XI.2014	1 ♀	N. Reiser
Pichincha	Quito	−0.2199, −78.5116	On house wall	10.XI.2014	1 ♂, 2 juv.	N. Reiser
Cotopaxi	Salcedo	−1.0446, −78.5902	House wall near Panamericana	14.XI.2014	1 ♂	N. Reiser
Tungurahua	Baños	−1.3970, −78.4231	Between drip rail/house wall	21.XI.2014	1 ♀	N. Reiser
Tungurahua	Ambato central	−1.2579, −78.6388	House wall	25.XI.2014	1 ♀, 1 juv.	N. Reiser
Tungurahua	Ambato-Montalvo	−1.3256, −78.6269	Wall	04.II.2018	5 ♀ , 1 ♂	J.F. Altamirano

Table 3. Global distribution of *Steatoda nobilis*. es = established and non-indigenous, ? = unclear status, ?? = unverified mentioning, pn = probably native, sp = single specimen record.

Country	Area	Literature	Status
Spain	Mainland	Denis 1957?; Melic 1994; Morano et al. 2018	es
	Balearic Islands	collection SMF	?
	Canary Islands	Pickard-Cambridge 1908; Schmidt 1990; Snazell and Jones 1993	pn
Portugal	Mainland	Snazell and Jones 1993; Morano et al. 2018	es
	Azores	Schmidt 1990; Wunderlich 1992; Borges and Wunderlich 2008	es
	Madeira	Thorell 1875	pn
France	South	Ledoux and Raphael 1998; Emerit and Ledoux 2013	es
	Corse	Canard 1989; Emerit and Ledoux 2013	es
	Atlantic coast	presented data	es
Italy	Mainland	Kulczycki et al. 2012	es
	Sicily	no published records	?
	Sardinia	Kulczycki et al. 2012	es
Great Britain		Pickard-Cambridge 1879; Snazell and Jones 1993	es
Ireland		Nolan 1999; Dugon et al. 2017	es
Germany		Table 1; Reiser 2013	es
Belgium		Van Keer 2010	?
Netherlands		Bink 2014; Van Helsdingen 2015	?
Turkey		Türkeş and Mergen 2005 (doubtful; see text)	sp
Iran		Zamani et al. 2015; pers. comm. Zamani	es
Morocco		Denis 1962	??
United States		Vetter and Rust 2012; Vetter et al. 2015	es
Chile		Taucare-Ríos et al. 2016; Ceryngier et al. 2018	es
Ecuador		Table 2; Faúndez et al. 2018	es
Colombia		Faúndez et al. 2018	es

(2017) showed that *S. nobilis* in Ireland seems to be restricted to man-made habitats and is currently not expanding into natural habitats such as forests or dunes. The habitat information based on about 400 records presented on the British Record Scheme website (British Spider and Harvestman Recording Scheme 2018) suggests a similar behaviour in Great Britain, but recently *S. nobilis* is spreading into semi-natural habitat

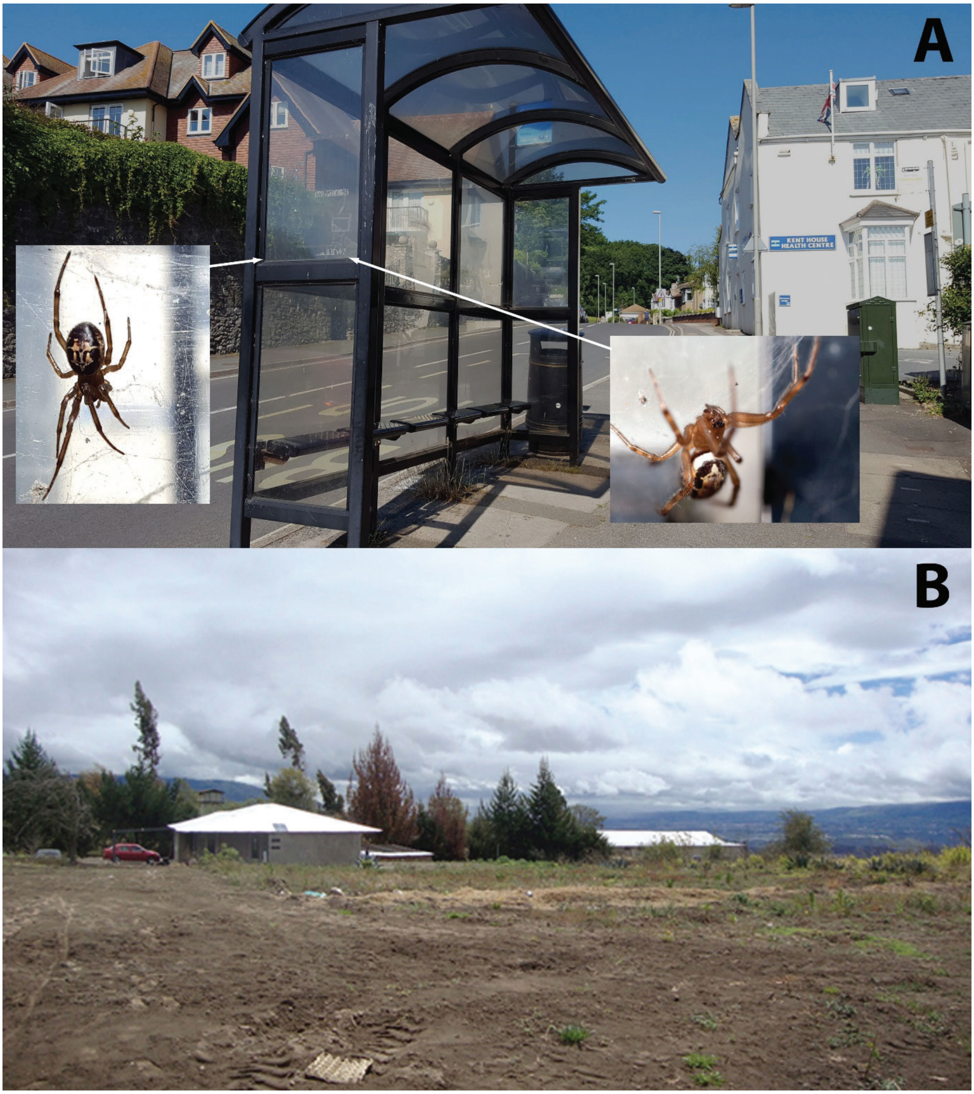

Figure 1. Diversity of habitats of *Steatoda nobilis* in its invasive range. **A** In the South of England, the species is not only abundant on stone walls and railings along the sea side, but also on man-made structures further inland, such as this bus stop in the coastal resort of Lyme Regis, Dorset, where its webs (inset) typically occur together with those of *Zygiella x-notata* (Araneidae) **B** Vicinity of the localities with records of *S. nobilis* in Ambato-Montalvo (Ecuador), August 2014.

in the southeast and is increasingly found in trees and scrubs in semi-natural habitats (P. Harvey pers. comm.).

In the Mediterranean, many of the *S. nobilis* observations were made in or around cities and villages (e.g. Kulczycki et al. 2012; Lecigne 2012; Melic 1994; Emerit and Ledoux 2013). However, several other specimens were collected in more natural habitats, e.g., a degraded pine forests near an urban area (Melic 1994), a (protected) marsh (Crespo et al. 2009), or dunes (Ledoux and Raphael 1998). An established population in Tehran, Iran (A. Zamani pers. comm.), is limited to urban areas.

In California (Vetter and Rust 2012; Vetter et al. 2015), most of the records were made randomly by citizens in their domestic environment and are therefore restricted to urban habitats, but it seems possible that *S. nobilis* is also spreading to more natural habitats in California. According to specimen data in the BOLD database (http://www.boldsystems.org/index.php/Public_BarcodeCluster?clusteruri=BOLD:ABA5272), five specimens of *S. nobilis* (1♀, 1♂, 3 immatures) were collected in 2011 at a dry dusty trail in Point Mugu State Park, south of Ventura, far from human habitation (specimen IDs BBUSE070-11, BBUSE071-11, BBUSE073-11, BBUSE105-11, BBUSE3156-12).

All records in Chile (Taucare-Ríos et al. 2016) and Ecuador were made in urban environments. The records in Ecuador may seem extraordinary, not just because of their localization in the inland of the country, but also because of the altitude of the records, at about 2600–2800 m for eight out of nine locations. However, records of *S. nobilis* in mountainous areas are known, e.g., from Spain (around 1000 m, Alicante; Morano et al. 2018) or Mount Teide, Tenerife (Schmidt 1968). Recently, Faúndez et al. (2018) published some records of *S. nobilis* from Ecuador collected in 2017 and 2018. Therefore, our specimens (Table 2) remain the oldest records of *S. nobilis* known from Ecuador.

On the Mediterranean mainland and in the Americas, over 50 % of all known localities are located within a distance of < 10 km to the coastline (Suppl. material 1: Fig. A5). This could be the result of several factors. Coastal regions often have the highest human population densities, which could result in more frequent collecting and reporting of *S. nobilis* by citizen scientists than in more rural areas (e.g. in California; Vetter et al. 2015). They are also often confronted with the highest propagule pressure of alien organisms due to long-distance trade and tourism, for example at the Italian localities discussed by Kulczycki et al. (2012).

All in all, *S. nobilis* seems to establish first in urban environments and is able to build up large populations in a short time. This generates further colonisation pressure on seminatural habitats in the environment of cities, finally leading to the establishment of populations outside the urban area.

Genetic structure of the New World populations of *S. nobilis*

To gain further insights into the most likely introduction routes of *S. nobilis*, we used publicly available DNA barcode sequences (~ 650 bp of the mitochondrial cytochrome c oxidase subunit I gene). We used barcodes for about 20 *S. nobilis* specimens from California and three specimens from Chile. The barcoded individuals are not identified to species in the BOLD database, but their distinct habitus, documented by photographs provided for many of the specimens, as well as their obvious abundance in synanthropic habitats in Southern California, nevertheless allow an unambiguous identification of the specimens. They form a single barcode cluster (BIN, BOLD:ABA5272, https://doi.org/10.5883/BOLD:ABA5272), without geographic structure, i.e. Chilean and Californian specimens share barcode haplotypes.

The genetic diversity of the Californian *S. nobilis* sample is relatively high, with an average pairwise distance of 0.78% and a maximum distance (within the Californian population) of 2.12%, compared to a median pairwise distance of 1.5% within *Steatoda* species with more than five published barcodes.

Dynamics of the expansion of *S. nobilis* in Great Britain

Additional insights into the changing invasive potential of *S. nobilis* can be gained from citizen science data obtained from the British Spider Recording Scheme. These show that *S. nobilis* is widespread in Great Britain and especially abundant in the southern half of the island, with scattered records along the northern coasts (British Spider and Harvestman Recording Scheme 2018; Suppl. material 1: Fig. A7). A comparison of the press coverage (number of articles in the Lexis-Nexis database which mention the scientific name) and the number of records in Britain show that both numbers closely correlate (Spearman's rank correlation, $p < 0.001$, $r = 0.78$) (Fig. 2). The total number of published press articles peaked in 2013 with 114 articles, followed by the highest amount of records in 2014 with over 150 observations of *S. nobilis* in Great Britain. In the following years, the number of records decreased slightly, in parallel with the number of articles. Examining the pattern of northward expansion of *S. nobilis* in Great Britain (Fig. 4), a long period of near-stasis following the initial establishment is quite notable; between 1984 and about 2010, despite thriving populations along the south coast and in the Thames estuary, no real change of the northern range boundary is evident at all (Fig. 4, Suppl. material 1: Fig. A6). Since about 2010, a persistent and accelerated expansion has occurred at a rate of at least 11 km/year when considering the contiguous range of the species and up to 95 km/year if the widely dispersed recent northernmost records are included (Fig. 4); the latter, however, are most likely the result of the long-distance transport of goods and materials and probably do not represent a real invasion front with established populations.

Niche exploration and distribution modelling

A principal component analysis of climate variables at native and invasive localities revealed that the two areas differ mainly in their annual temperature range and seasonality (Suppl. material 1: Fig. A1, Table A3). With the exception of the invasive locations in Ecuador, the native area shows a lower temperature range than the invasive area. A global prediction of potential occurrences of *S. nobilis* (Fig. 3) shows a relatively restricted area of suitable localities for the further spread of the species. In Europe, besides the known invasive areas the model especially predicts north and western parts of France and some coastal areas of the North Sea as highly suitable. Some islands in the

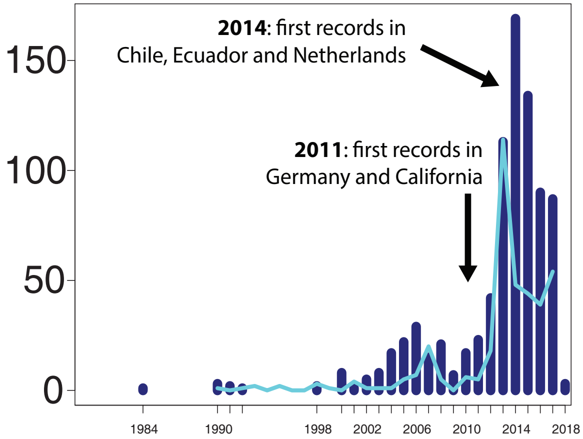

Figure 2. Increase in Spider Recording Scheme records for *Steatoda nobilis* in Britain parallels intensified press coverage in the local newspapers (dark blue bars = spider records, light blue line = number of press articles). The sudden massive increase in records seen in the last decade coincides with the first appearance of the species in various other countries far from the native range in the Macaronesian islands.

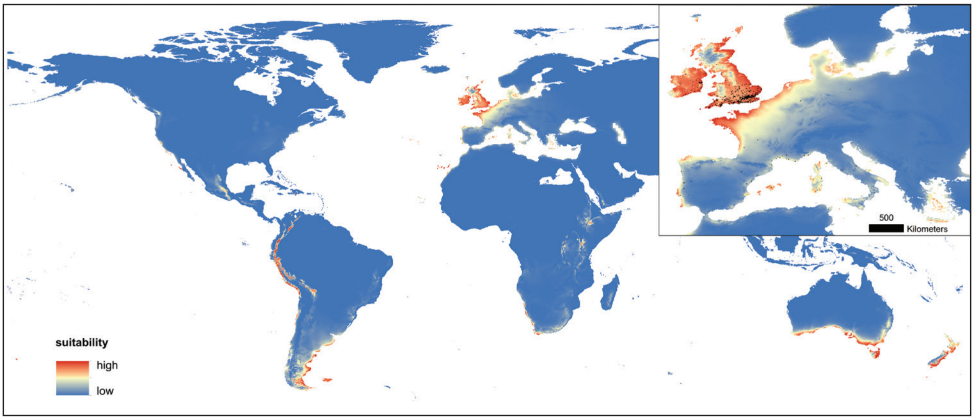

Figure 3. Global climatic suitability of *Steatoda nobilis*. Mean Maxent prediction, black dots in insert depict known invasive populations in Europe (insert is not masking suitable areas).

Aegean Sea and especially Crete might also be very suitable for *S. nobilis*. On a global scale, especially coastal regions with constant temperature regimes were predicted as suitable, e.g., in southern Australia and New Zealand.

To test our predictions, we conducted a field trip to Granville, Normandy (France). The coastline of the Normandy is predicted as a highly suitable area for *S. nobilis* by our model and has an oceanic climate similar to the south coast of England. Contrary to the latter, no populations of *S. nobilis* are yet known from the northern Atlantic coast of France near to the Channel Islands. Nevertheless, we were able to locate a local *S. nobilis* population in the city area of Granville and collected a single adult female (Fig. 5A) together with an egg sac behind a street sign next to a paved boardwalk (17.

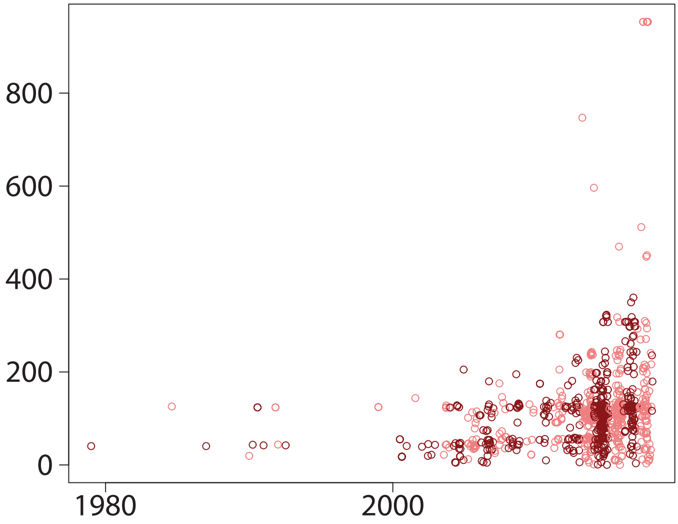

Figure 4. Northward distance (km) of recent records of *Steatoda nobilis* in Great Britain. Distances are calculated relative to the first known record in the country in Torquay, based on data in the SRS database and in Snazell and Jones (1993). Data from consecutive years are alternatingly coloured dark and light red.

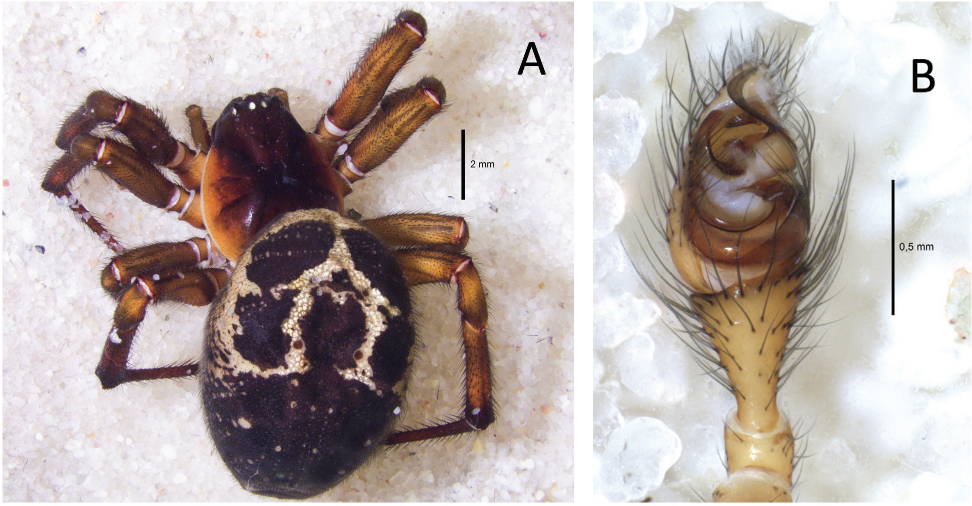

Figure 5. Habitus and male genitalia of *Steatoda nobilis*. **A** Female from Normandy, France **B** Male pedipalp in ventral view from Stuttgart, Germany.

IX.2018, 48.8331N, -1.5879W, T. Bauer leg.). Webs of the species could also be found between sea defence rocks directly at the coastline (48.8342N, -1.5929W) as well as on trees in a small urban forest fragment (48.8334N, -1.5892W) next to the location of the female. Around 100 spiderlings emerged from the egg sac in captivity.

Discussion

Probable introduction pathways of *S. nobilis*

Steatoda nobilis seemed unable to establish viable populations in either Great Britain or Germany at the time of copious and untreated banana imports (= highest propagule pressure; Allendorf and Lundquist 2003). Although it is often suggested or indicated that the British *S. nobilis* populations are descendants of specimens imported with fruits from the Canary Islands (e.g., Roberts 1995; Kulczycki et al. 2012), this hypothesis is not well supported by current data. Since the 1960s, different phytosanitary treatments and transport techniques have become standard procedure in international trade and dramatically reduced the amount of alien spiders transported to Europe via shipping, especially after 1971 (Hallmann 2007; Nentwig 2015). Flourishing British populations of *S. nobilis* were not found until 1986 (Portsmouth area), followed by subsequent records and the observation of rapid colonization events (Snazell and Jones 1993). Great Britain has a long tradition in field arachnology, and it seems very unlikely that in the times of Bristowe (1941) and Locket and Millidge (1951, 1953, 1974) large populations of *S. nobilis* in domestic areas and harbours of the South of England would have been missed, especially as the urban and synanthropic spider fauna of Britain has traditionally received much closer attention than in other countries (e.g., Bristowe 1929, 1939). Therefore, it cannot be excluded that other introduction pathways, e.g., accidental imports by tourists (the Canary Islands are traditionally visited by British tourists every year, with several millions in 2016 alone (Patronato de Turismo Gran Canaria 2017)), specimens (or even populations) on returning cruise ships from Macaronesia, or the import of ornamental plants at least contributed to the establishment of *S. nobilis* in Great Britain.

The populations found in Cologne described in this work are most probably descendants of specimens introduced with potted plants. Very large amounts of cacti and other succulents are imported every year from the Canary Islands (e.g., by the grower "Canary Cactus"), but Kulczycki et al. (2012) pointed out that *S. nobilis* also achieves high population densities in Italian plant nurseries. Ornamental plants sold in garden centres in Germany are normally of various origins (e.g., Canary Islands, Iberian Peninsula, Dutch wholesalers); therefore, it seems currently impossible to determine the exact source of the German populations. Because we and Reiser (2013) only found scattered records/populations of *S. nobilis* among a large number of non-colonized garden centres (e.g., only two out of about 10 garden centres were colonized in Cologne), it can be tentatively concluded that *S. nobilis* is currently restricted to very few localities in Germany and that garden centres in Central Europe seem to be a potential habitat for this species.

In the Netherlands, a record from the Maasvlakte (Bink 2014) was possibly the result of an accidental introduction with armour rock for sea defences. Another record (juvenile female) from the Netherlands was made on 19 May 2016 at the coast in the Southwest (51.5879, 3.5667, leg. F. van de Putte, deposited at Naturalis Leiden after its final moult) under the bark of a tree in the neighbourhood of several camping areas, to which it was possibly introduced by tourists from France or England.

In South America, it seems possible that *S. nobilis* has been spread and/or is distributed by human assistance along the Panamericana Highway (the most important inland transportation route in South America), as two of the Ecuadorian locations (Ambato and Salcedo) are crossed by the route, and Temuco in Chile is situated on one of the two main southern branches of the route.

Distribution and outlook of *S. nobilis*

The climate exploration and the ability of the distribution model to explain the current distribution of *S. nobilis* with climate variables revealed that the general distribution is not decoupled from the regional climate. Even though most alien records of *S. nobilis* were located in urban environments, there is currently much evidence that the species is not restricted to urban areas and has already or will spread into seminatural or natural habitats in the invaded areas, e.g., in the South of England and France (Ledoux and Raphael 1998; P. Harvey pers. comm.). Additionally, if only local microclimates were relevant for the distribution of *S. nobilis*, we would expect a much more random establishment, similar to *Steatoda grossa*, another large theridiid, living mostly indoors in large parts of its cosmopolitan distribution (Heimer and Nentwig 1991; Le Peru 2011; World Spider Catalog 2018). We note that our modelling approach and the resulting map is a rather conservative prediction, which probably underpredicts the true potential distribution. SDMs calibrated in early stages of an invasion tend to underestimate the potential distribution, as not all suitable conditions are occupied yet and, therefore, only a part of the species' niche is captured by the model (Václavík and Meentemeyer 2012). However, the most recent findings of this species at locations (not used for modelling) in Normandy, France (Table 3, Fig. 5a) and Bogotá, Colombia (Faúndez et al. 2018), which were predicted as highly suitable by our model, corroborate the usefulness of our predictions. Our SDM identified several areas characterized by an oceanic climate on nearly all continents as habitat for *S. nobilis*. Especially parts of South Africa, the southern coastline of Australia and wide parts of New Zealand are highly suitable for *S. nobilis*. All three countries are heavily afflicted by invasive species (e.g., Hoffmann and Broadhurst 2016; Paterson et al. 2011; Aikio et al. 2010), and an invasion of *S. nobilis* could have unpredictable and severe consequences for the native fauna.

The rapid east–west spread of *S. nobilis* in Britain, followed by a much later substantial northward expansion especially since 2010, indicates that the range of *S. nobilis* initially was not constrained by limited dispersal ability. Interestingly, between 1984 and 2010 there was a period of well-known range expansions in other species (Parmesan and Yohe 2003). Recently, these ecological limitations seem to have been overcome rather suddenly by *S. nobilis*, leading to a rapid increase in population number and an expansion far beyond its original centres in the UK. The first period of stasis between 1985 and about 2010 can be interpreted as a typical lag-phase (a phase with little or no spread in the new environment), a phenomenon observed in many invasive species (Kowarik 1995; Crooks and Soulé 1999). What could have caused this accelerated spread? The northward expansion of adventive invertebrate species in Europe, and especially in the UK, is often attributed to the effects of climate change. However, the extent, unpredictability, and sudden rapidity of the expansion of *S. nobilis* all over the globe make this explanation unlikely. There is no indication that climate conditions across the UK have changed dramatically enough in the last 20 years, to turn much of the UK into favourable habitat for an originally Macaronesian species. Climate models for other species show a much more restricted expansion of the accessible range for this relatively brief period (e.g., Lundy et al. 2010), and such a more limited expansion would also agree with predictions made by climate modelling (Loarie et al. 2009). Also, the expansion persisted unimpeded through the very cold winter of 2010, while severe weather events like this would have halted and most likely reversed an expansion that was merely the result of global warming, as shown by Tinsley et al. (2015) for alien *Xenopus laevis* populations in Britain and predicted by Avery et al. (2010) for range expansions of alien Burmese pythons, *Python bivittatus*, outside of subtropical areas in the United States.

Could the observed distribution trends in Great Britain just be the result of greater awareness and thus more intense reporting, rather than of actual population growth and range expansion? Fig. 2 indeed shows that newspaper coverage and the number of *S. nobilis* records in the UK correlate quite closely. However, the causality seems to work both ways, with increased awareness contributing to some increase in reporting (although most non-expert reports of *S. nobilis* refer to a wide diversity of common house spiders, rather than actual false widows; Bee 2013; P. Harvey pers. comm.), while sustained and widespread coverage became only possible once the species had become common enough through large parts of the most populated areas of the UK leading to a larger number of reportable encounters. Moreover, the sudden expansion of the *S. nobilis* in the UK, after many years close to stasis, closely coincided with a wave of newly established populations in widely distant countries (Fig. 2) and a sudden rise of reports in Irish online spider groups (Dugon et al. 2017). In general, this indicates that the expansion is not a simple collection artefact based on increased awareness of naturalists and the public (Aikio et al. 2010), as the spread of *S. nobilis* was observed in different countries in the same time period and by different groups of naturalists and laypeople. Such lag-phases are also sometimes caused by a low number of specimens and populations connected to a slow growth rate in the beginning of an invasion (Crooks and Soulé 1999). After reaching a critical point in the exponential growth, the species becomes common and is spreading. However, this explanation seems unlikely because the first established populations of *S. nobilis* in Southern England were described as “flourishing” (Snazell and Jones 1993), indicating a high population density 30 years before the northward spread of the species. Together with the ability to build several egg sacs in one season (Snazell and Jones 1993), the colonisation pressure caused by the first *S. nobilis*-populations was probably very high already in the early years after the establishment.

Another possible reason for the spider's expansion success is evolutionary adaptation, e.g., an ecological niche expansion (Guisan et al. 2014) in the British populations. This has been suggested in *Argiope bruennichi* (Scopoli, 1772) and *Cheiracanthium punctorium* (Villers, 1789), two spider species which have naturally expanded their distribution in the last decades (Krehenwinkel and Tautz 2013; Krehenwinkel et al. 2015, 2016). The emergence of such adaptations can require extended times, leading to a characteristic lag phase of range expansion (Lee 2002; Aikio et al. 2010). Adaptive genetic variants could have entered the invasive populations by novel mutations (Jensen 2014), as part of the standing variation of native populations (Barrett and Schluter 2008), or by admixture of formerly isolated lineages in the course of the invasion (Nolte and Tautz 2010). However, to rigorously test the possibility of evolutionary adaptation driving the species' expansion, ecological experiments, e.g., reciprocal transplants and thermal tolerance tests (Krehenwinkel and Tautz 2013; Krehenwinkel et al. 2015), will be necessary.

The obvious prediction resulting from this assessment and our model is that the expansion of *S. nobilis* is likely to continue rapidly in the coming years. The western Mediterranean islands, parts of South Africa, southern Australia, and New Zealand will face the highest risk of colonisation in the future. It is possible that some of these areas, especially the western Mediterranean islands, are already inhabited by *S. nobilis* and the species has been overlooked, similar to the newly reported population in the Normandy, France. An intensified monitoring and search for *S. nobilis* in inhabited areas and regions predicted as suitable could therefore reveal the real distribution of the species.

As the DNA barcoding data indicate that *S. nobilis* is genetically very distinct from its congeners, it is unlikely that the species will hybridize with native species. This was observed for alien *Latrodectus hasselti*, which hybridized at least in one population with a native *Latrodectus* species in New Zealand (Vink et al. 2008). The barcode data also indicate that established populations in the New World are likely the result of multiple introductions (c.f. Kolbe et al. 2004), as the observed haplotype diversity (five distinct haplotypes with an average distance of 0.81%) seems inconsistent with a severe recent population bottleneck (e.g., a single founder female) for the populations in California and Chile. Similar deep genetic diversity is seen for populations of other invasive theridiids, such as *Parasteatoda tepidariorum* (BOLD:AAC0175, average distance=1.21%, maximum distance (globally)=3.61%, *n*=222) and *Steatoda grossa* (multiple BINs, *n* = 43), while *Nesticodes rufipes* (BOLD:AAG4814, average = 0.24%, maximum = 1.01%, *n* = 22) shows a much more homogeneous barcode gene pool. In comparison, 2009 specimens of the recently expansive *Argiope bruennichi* (BOLD:AAJ1655) (Araneidae) show an average barcode distance of only 0.37% (maximum = 2.63%), and 295 specimens of the invasive *Cheiracanthium mildei* (BOLD:AAB7601) (Cheiracanthiidae) are only slightly more diverse (average = 0.43%, maximum = 1.21%), thus exhibiting considerably less diversity than seen in the New World populations of *S. nobilis*. Nevertheless, *S. nobilis* competes with other spider species (Kulczycki et al. 2012) and could outcompete native species with a similar niche on the local scale and possibly threatens other arthropod species due to predation, similar to *Latrodectus hasselti* in New Zealand

(Bryan et al. 2015). It was observed that *S. nobilis* preys on a protected reptile species in Ireland (Dunbar et al. 2018), which shows that the spider is able to subdue small vertebrates. *Steatoda nobilis* can produce large amounts of offspring for a long period after mating, and adult females are extraordinarily long-lived and persistent (Locket 1979; Snazell and Jones 1993). Snazell and Jones (1993) reported on a female which lived for 5½ years in captivity. Therefore, an eradication of established populations seems impossible, especially as juvenile specimens are very hard to locate due to their cryptic lifestyle in crevices and holes (Snazell and Jones 1993; Kulczycki et al. 2012), and many populations are probably supported by repeated introductions, as shown by the barcode data. Additionally, their ability to balloon (Kulczycki et al. 2012) enables this species to overcome natural and anthropogenic barriers (e.g., rivers, streets) and to distribute over relatively large areas in a comparatively short time. Adjusted phytosanitary treatments of potted plants could potentially decelerate the spread of *S. nobilis* (and other alien spider species). As documented for Britain, the species is also able to cause public disruption and high economic costs in invaded areas, e.g., by increased consulting of pest management services in private homes or the closing of public institutions. Together with the potential ecological consequences of a further spread, *S. nobilis* has to be considered as one of the most invasive spider species in the world.

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Supplementary material 1

Dataset compilation, niche exploration, species distribution modelling, and additional analyses

Authors: Tobias Bauer, Stephan Feldmeier, Henrik Krehenwinkel, Carsten Wieczorrek, Nils Reiser, Rainer Breitling

Data type: occurences, analyses, background information

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The interplay between propagule pressure, seed predation and ectomycorrhizal fungi in plant invasion

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Abstract

There are many hypotheses aiming to explain invasion success, but evaluating individual hypotheses in isolation may hinder our ability to understand why some species invade and others fail. Here we evaluate the interaction between propagule pressure, seed predation and missed mutualism in the invasion success of the pine, *Pinus ponderosa*. We evaluated the independent and interactive effects of propagule pressure and seed predation at increasing distances from a pine plantation. Additionally, because pines are obligate mutualists with ectomycorrhizal fungi (EMF) and pine invasions fail in the absence of their EMF symbionts, we evaluated EMF availability through a growth chamber bioassay. In this bioassay we measured root colonization by EMF with soil samples collected from the different distances from the plantation. We found that propagule pressure overwhelms seed predation only at the edge of the pine plantation, while seed predation overcomes propagule pressure at 25 m and further distances from the plantation. We also found that EMF root colonization decreases with distance from the plantation. However, pine roots were colonized up to 200 m from the plantation, suggesting that EMF may not be hindering invasion, at least not on the scale of this experiment. Taken together our results demonstrate that seed predation may be limiting the invasion of *P. ponderosa* in the study region as propagule pressure only overcomes seed predation at the plantation edge. Here we provide evidence of how strong biotic resistance can suppress an invasion, regardless of the variation in propagule pressure and the availability of mutualists.

Keywords

Biotic resistance, granivory, invasion mechanism, mutualism, *Pinus*, seed dispersal

Introduction

For decades, ecologists have tried to explain why some populations of introduced species become invasive while others fail to invade (Elton 1958, Lockwood et al. 2013). Numerous hypotheses seek to explain the mechanisms behind biological invasions (Catford et al. 2009). The propagule pressure hypothesis posits that increasing the number of individuals released in a region where they are not native increases the probability of invasion success (Lockwood et al. 2005). This hypothesis seems to be more supported by empirical evidence than most other hypotheses proposed in invasion biology (Simberloff 2009, Lamarque et al. 2011, Blackburn et al. 2015) and, therefore, may be the hypothesis carrying the greater consensus (Jeschke 2014). Another major hypothesis in biological invasions is the biotic resistance hypothesis (Elton 1958), which states that competitors, predators, herbivores and pathogens in the resident community reduce invasion success (Levine et al. 2004). Seed predation is one of the major filters for plant establishment (Janzen 1969, Hulme 1994, Larios et al. 2017) and, consequently, a major barrier to plant invasions (Nuñez et al. 2008, Pearson et al. 2011, Connolly et al. 2014). Studies on the interplay between propagule pressure and biotic resistance found that the influence of propagule pressure on invasion success is more important for high levels of biotic resistance (von Holle and Simberloff 2005, Jones et al. 2017).

Together with strong biotic resistance and low propagule pressure, the absence of mutualistic biotic interactions (missed mutualism) is one of the most important limitations for the invasion of non-native plants (Traveset and Richardson 2014, Dickie et al. 2017). More than one third of the most widespread invasive woody species form mutualistic interactions with mycorrhizal symbionts, more than two thirds form interactions with seed dispersers and the vast majority of invasive woody plants are animal pollinated (Traveset and Richardson 2014). If a non-native plant needs an obligate mutualist its absence in the invaded range will act as a strong filter to its invasion success (Traveset and Richardson 2014, Dickie et al. 2017). Even though the importance of different mechanisms is recognized in invasion processes, most studies on biological invasions focus on a single hypothesis without considering the possible interaction between different mechanisms. As a consequence, we lack an understanding of how different processes interplay to determine invasion success or failure (Pearson et al. 2018).

Pines are an ideal system to study the role of propagule pressure, seed predation and missed mutualism on invasion success. The role of propagule pressure can be easily studied because non-native pines produce a large number of seeds which are wind dispersed over large areas but with the highest proportion falling near the seed source, creating a gradient of propagule pressure (Nathan et al. 2011). The role of biotic resistance, through seed predation, can be assessed because seeds of non-native pines are highly consumed by native generalist seed predators (Nuñez et al. 2008, Chiuffo et al. 2018). Additionally, pines are obligate mutualists with ectomycorrhizal fungi (EMF) and pine invasion fails in the absence of their EMF symbionts (Nuñez et al. 2009), thus the effect of missing mutualism can be straightforwardly evaluated using pines. Furthermore, EMF are dispersed from the invasion source, creating a gradient of decreasing abundance of fungal symbionts (Collier and Bidartondo 2009, Hayward et al. 2015, Horton 2017).

The aim of this study was to test the importance of three mechanisms (propagule pressure, seed predation and missed mutualism) in the invasion of *Pinus ponderosa* in north Argentinean Patagonia. Here, we evaluated seed predation with different seed availability to resemble the natural seed rain pattern at different distances from a plantation of *P. ponderosa*. Additionally, to test if the absence of highly co-evolved soil mutualists could account for pine invasion failure we performed a growth chamber bioassay with soil inoculum from increasing distances from the pine plantation. Specifically, we asked the following question: what is more important determining *P. ponderosa* invasion success, propagule pressure, seed predation, the availability of EMF symbionts or the interplay between these mechanisms? Evaluating the relative importance of different invasion mechanisms is key to identify the causes of plant invasions. Also, assessing the interaction between different mechanisms helps to understand how different processes interplay to determine invasion success or failure (Pearson et al. 2018).

Methods

Study area

We conducted this experiment on a steppe ecosystem in North Patagonia (40°59'53"S, 71°05'13"W) because this type of ecosystem is among the most frequently invaded by non-native pines (Richardson et al. 1994). In Patagonia, large areas of steppe have been replaced by pine plantations during the past few decades (CIEFAP 2017), which are now seed source for invasions. *Pinus ponderosa* is the most widely planted species in the region, covering over 90% of the forestry area (CIEFAP 2017). However, *P. ponderosa* is only rarely invasive in this region (Sarasola et al. 2006). Post dispersal seed predation for pines in this system is largely carried by the native rodents *Oligoryzomys longicaudatus* and *Eligmodontia morgani* (Chiuffo et al. 2018). There is no evidence of secondary seed dispersal of *P. ponderosa* by animals in the study region (Nuñez et al. 2008, Chiuffo et al. 2018). On the study site, mean annual rainfall is 580 mm, concentrated during fall and winter (March-September), and mean annual temperature is 8.6 °C (San Ramon ranch meteorological station). Vegetation cover is ca. 60% being *Pappostipa speciosa* and *Festuca pallescens* the dominant plant species (Anchorena and Cingolani 2002).

Propagule pressure vs. seed predation

To evaluate the effects of propagule pressure and seed predation on seed survival, we conducted a seed predation experiment. We placed 18 transects every 50 meters perpendicular to a *P. ponderosa* plantation. In each transect, we installed plots at 0, 25, 50, 75, 100, 150 and 200 m from the plantation. To disentangle the effects of distance from plantation and seed availability, we used two different experimental designs with different seed densities (variable and fixed). In the "Variable density" experiment we simulated natural seed dispersion on nine transects. The purpose of the "Variable density" experiment is to evaluate the proportion of seed rain that is consumed by seed predators at different distances from the pine plantation. Since data on seed production and dispersion is not available in our study site, we obtained data from the literature (Barrett et al. 1979, Krannitz and Duralia 2004). Specifically, we used data on the percentage of seeds that reach different distances from the edge of a *P. ponderosa* plantation and built a regression model that best described the variation in seed dispersal with distance from plantation. To fit this dispersion kernel we used a negative exponential model because it is appropriate for describing seed dispersal by wind (Clark et al. 2005, Lustenhouwer et al. 2017). Based on the dispersion kernel of this species we placed 100, 50, 30, 16, 10, 6 and 2 seeds per m^2 at 0, 25, 50, 75, 100, 150 and 200 m from the plantation respectively (Fig. 1). In the "Fixed density" experiment we put 20 seeds per m^2 at 0, 25, 50, 75, 100, 150 and 200 m from the plantation at each of the nine transects. We chose to put 20 seeds per m^2 because it represents an intermediate value between the mean (ca. 30 seeds/m^2) and the median (16 seeds/m^2) of the seed rain across all the distance levels. The purpose of the "Fixed density" experiment is to evaluate the effect of the distance from the pine plantation on seed predation. We glued the seeds to popsicle sticks with a non-toxic odor-free adhesive and we fastened sticks to the ground with stakes to prevent the removal of the sticks by seed predators. We used latex disposable gloves to handle the seeds to avoid imparting human odor to the seeds. We considered seed removal as evidence of seed predation (Nuñez et al. 2008, Chiuffo et al. 2018). In total, we placed 2013 seeds and 1473 sticks. We carried out this study during autumn because it is the period when pine seeds are naturally available (Krugman and Jenkinson 2008) and when rodent abundance tends to peak (Guthmann et al. 1997). We evaluated seed removal 30 days after installing the experiment.

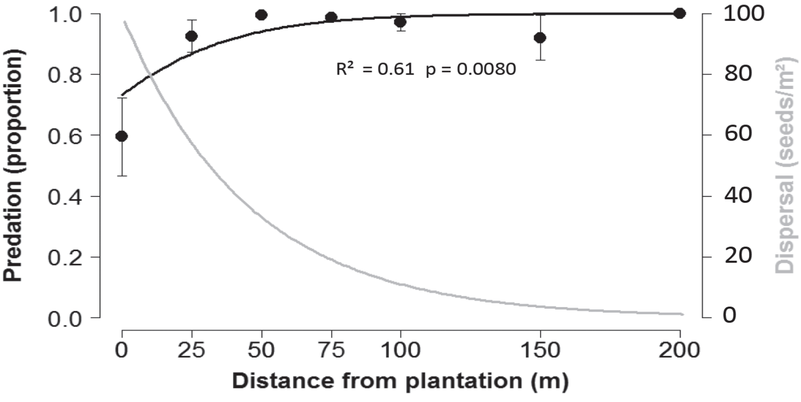

Figure 1. Seed dispersal (grey solid line) and seed predation (black dots and black solid line) of *P. ponderosa* at increasing distance from plantation. Dots show mean (+ SE) values of seed predation for each distance.

Missed mutualism

To test if lack of ectomycorrhizal fungi is limiting *P. ponderosa* invasion, we conducted a growth chamber bioassay. We collected ~100 cm^3 of soil from each of the seven distances (0, 25, 50, 75, 100, 150, and 200 m) from the plantation on each of the nine "Variable density" transects used for the field experiment. Soils were collected during late fall 2017, just after the peak of mushroom fruiting season. Soil samples were dug from each site using an ethanol-sterilized spoon, placed in coin envelopes, each of which was then placed into gallon bags and then stored at 4 °C to be used the next day after collection. We removed small rocks and coarse roots and we used these soils to inoculate a mix of sterilized 50:50 soil and sand that had been autoclaved twice (Wolf and Skipper 1994). We randomly distributed soils from different distances into pots of 12 cm depth in ethanol sterilized plastic trays. To detect inadvertent EMF inoculation in the growth chamber, we randomly located 12 pots filled only with sterile soil. We used a total of 75 pots (nine for each from seven distances and 12 sterile) randomly distributed in five planting trays that were rotated once a week inside the growth chamber to avoid location effects. In each pot we planted four *P. ponderosa* seeds. We bought *P. ponderosa* seeds in the National Institute of Agricultural Technology (INTA – Bolson Experimental Station). We performed a pre-germination treatment in cold water for 48 hours. Floating seeds (vain seeds) were discarded and the rest were stored at 2–5 °C for three weeks. Prior to being sown, we surface-sterilized seeds in a 1% sodium hypochlorite solution. During the experiment water was added *ad libitum*, and there were no nutrients added to the pots. Light and temperature were kept constant. We used a cycle of 12 hours of light and 12 hours dark. Temperature was 25 °C during light periods and 10 °C during dark periods. Only the first plant to emerge was left in the pot, the rest were cut at the soil level avoiding soil disturbance. During the growing period we recorded survival (number of seedlings alive/total seedlings) for each distance and for sterile controls. After six months of initial planting we harvested the plants. We measured shoot height. We carefully rinsed clean seedling of adhering soil, separated them at the soil line into a root and shoot compartment, and placed them into an envelope to be dried in an oven at 65 °C for 2 days. We measured the biomass of dried root and shoot fractions separately using an electronic balance with accuracy to 0.0001 g. Prior to drying, we carefully examined the root system of each *P. ponderosa* seedling under a dissection microscope to address the extent of ectomycorrhizal colonization (based on morphological characteristics). Roots were placed on a petri dish, and the number of fine root tips colonized and not colonized by ectomycorrhizal fungi recorded.

Statistical analysis

To evaluate the effect of different predictive variables on seed predation we used logistic regressions. We calculated seed predation, the response variable, as the proportion of seeds that had been consumed (Orrock et al. 2015). To determine if distance from plantation and transect type had a significant effect on seed predation we considered them as fixed factors. Because our experimental design included experimental units nested in transects we used transect number as a random factor (Bolker et al. 2009). To evaluate if the relationship between seed predation and distance from plantation differed between fixed and variable density transects we included the interaction between variables "distance" and "transect type" in our models. To compare seed predation between different distance levels we built logistic models with seed predation as response variable and distance level as a fixed categorical variable. Then we compared seed predation of each distance level with all the rest distance levels. To compare propagule pressure with seed predation at each distance level we also built logistic models with distance level as a fixed categorical variable. In this case we included a "dummy" distance level with 100% seed predation (proportion equal to 1) to represent propagule pressure. Then we compared seed predation of this "dummy" distance level with seed predation at distance levels 0, 25, 50, 75, 100, 150 and 200 m from the pine plantation. Significant differences would indicate that propagule pressure is higher than seed predation, while no significant differences would indicate that seed predation was as high as propagule pressure. For all our models we assumed a binomial distribution, using Generalized Linear Mixed Models (GLMM) based on Laplace approximation and a logit link function (lme4 package, glmer function) (Bates et al. 2015). We used analysis of deviance to evaluate the amount of total variation explained by each of the fixed factors (pseudo R^2).

To evaluate the effect of mycorrhizal inocula at increasing distances from plantation, we analyzed each response variable (survival, shoot height, biomass, and root colonization) separately. For response variables with binomial distribution (survival, and root colonization) we used GLMM fit by maximum likelihood and a logit link function (lme4 package, glmer function) (Bates et al. 2015). For those variables with a binomial distribution that presented overdispersion, we included an observation-level random effect for modeling the overdispersion (Harrison 2014). For response variables with normal distribution (shoot height and biomass) we used linear mixed-effects model fit by residual maximum likelihood (REML) (nlme package, lme function) (Pinheiro et al. 2017). In all cases we included "distance" as a fixed factor and "transect" as a random factor in the model. To address the effect of root colonization in plant growth we compared aboveground biomass (shoot dry biomass) of colonized vs. uncolonized seedlings with ANOVA (at $\alpha = 0.05$). All analyses were performed with R 3.4.0 statistical software (R Core Team 2018).

Results

Propagule pressure vs. seed predation

We found that seed predation increased with distance from plantation ($p = 0.0080$, Fig. 1, Table 1, 2). Propagule pressure was higher than seed predation only at the plantation edge (0 m) and seed predation surpassed propagule pressure at distances of 25 m and further from the plantation (Table 3). Seed predation was between 30 and 40% lower at the plantation edge than at distances of 25 m and further from the plantation (Fig. 1).

Table 1. Parameters of the logistic regression for seed predation of *P. ponderosa* at increasing distance from the pine plantation.

Fixed effects of distance from plantation, transect type and the interaction between distance and transect type. Significant effects ($p < 0.05$) are shown in bold letters				
Fixed effects	**Estimate**	**Standard error**	**z**	**p**
Distance	0.036	0.0134	2.656	**0.0080**
Transect type	-0.849	0.7791	1.090	0.2756
Distance * Transect type	-0.015	0.0203	0.731	0.4648
Random effects of the transect (n = 9). Intercepts are averaged				
Random effect	**Mean intercept**		**Standard deviation**	
Transect	1.475		0.000	

Table 2. Pairwise comparisons of seed predation levels between different distances from the pine plantation. Significant differences ($p < 0.05$) are shown in bold letters.

Pairwise comparison [m]	p value
0 vs 25	**0.0396**
0 vs 50	**0.0397**
0 vs 75	**0.0312**
0 vs 100	**0.0291**
0 vs 150	**0.0441**
0 vs 200	**0.0438**
25 vs 50	0.4273
25 vs 75	0.5187
25 vs 100	0.6585
25 vs 150	0.9311
25 vs 200	0.4090
50 vs 75	0.8348
50 vs 100	0.6705
50 vs 150	0.3914
50 vs 200	0.9541
75 vs 100	0.8175
75 vs 150	0.4724
75 vs 200	0.7925
100 vs 150	0.6011
100 vs 200	0.6359
150 vs 200	0.3758

Table 3. Pairwise comparisons between propagule pressure and seed predation for each distance level from the pine plantation. Significant differences ($p < 0.05$) are shown in bold letters.

Pairwise comparison	Distance [m]	p value
Propagule pressure vs seed predation	0	**0.0438**
	25	0.4090
	50	0.9541
	75	0.7925
	100	0.6359
	150	0.3758
	200	1.0000

Moreover, we found no effect of transect type (variable vs. fixed) on seed predation ($p = 0.2756$). The relationship between seed predation and distance from plantation was the same for both transect types as evidenced by the absence of interaction between distance from the plantation and transect type ($p = 0.4648$). With the fixed density transects we offered 25%, 100%, 230% and 900% more seeds than would naturally be available at 75, 100, 150 and 200 m from the plantation, respectively, but still seed predators consumed 97%, 98%, 88% and 100% of the seeds dispersed, respectively.

Missed mutualism

EMF root colonization decreased with distance from plantation (p = 0.0139, Fig. 2, Table 4). Mean EMF root colonization at the pine plantation edge was ca. 90% while it decreased to ca. 40% at the furthest distance from the plantation evaluated here (200 m). However, we found no differences in seedling growth (shoot height and biomass) or survival with distance from plantation ($p_{height} = 0.6387$; $p_{biomass} = 0.9911$; $p_{survival} = 0.4830$). When we pooled all distance levels together and compared seedlings colonized by EMF (ranging from 10% to 100% EMF root colonization) with seedlings un-colonized (0% EMF root colonization) we found differences in seedling biomass favoring colonized seedlings (p = 0.0400, Suppl. material 1: Fig. S1). Finally, the roots of the seedlings growing in sterile soils were not colonized by EMF, showing there was no inadvertent EMF inoculation in the growth chamber.

Table 4. Parameters of the logistic regression for EMF root colonization at increasing distance from the pine plantation.

Fixed effects of distance from plantation. Significant effects (p < 0.05) are shown in bold letters				
Fixed effects	**Estimate**	**Standard error**	**z**	**p**
Distance	-0.012	0.0050	-2.460	**0.0139**

Random effects of the transect (n = 9), and the observation included due to overdispersion. Intercepts are averaged		
Random effect	**Mean intercept**	**Standard deviation**
Transect	2.064	0.396
Observation	2.027	1.554

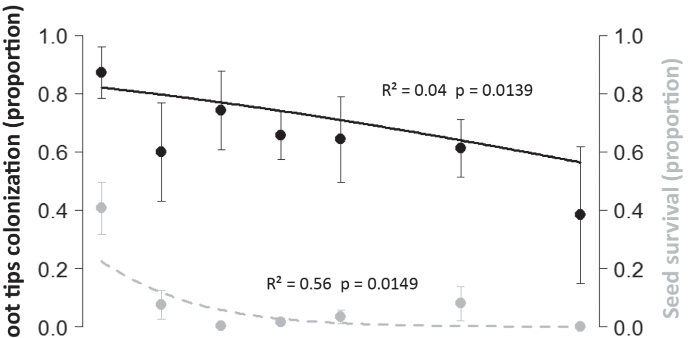

Figure 2. EMF root tips colonization on growth chamber bioassay (in black) and seed survival from predation for *P. ponderosa* on field experiment (in grey) at increasing distance from plantation. Dots show mean (+ SE) values for each distance.

Discussion

Our results provide strong empirical evidence that seed predation may be the most important biotic mechanism limiting *P. ponderosa* invasions in this system. Seed predators consumed ca. 95% of the seeds dispersed outside the pine plantation, thereby limiting seed availability. Propagule pressure varied in a wide range, from 100 seeds/m^2 at the plantation edge to 2 seeds/m^2 at a distance of 200 m from the plantation, however, only at the plantation edge does propagule pressure overwhelm seed predation. At distances of 25 m and further from the pine plantation, seed predation overcame the influence of propagule pressure. This is particularly clear when we consider seed predation in the fixed density transects: at distances from plantation higher than 75 m seed predation was between 25% and 900% higher than propagule pressure. This survival pattern of not predated seeds suggests that *P. ponderosa* seedling annual recruitment is limited to the first 25 m from the seed source. Altogether, these results provide evidence of how biotic resistance from generalist natural enemies can hinder an invasion.

In this study, we found that pine seed predation probability increases with increasing distance from plantation (Fig. 1). This fits with the seedling recruitment pattern described by McCanny (1985). Such inverse density-dependence seed mortality can be explained by predator satiation (Janzen 1971), when predators are satiated by the higher seed densities near adult plants (Augspurger and Kitajima 1992). Predator satiation near the pine plantation makes sense when we consider the gradient of decreasing seed availability with increasing distance from the plantation (Fig. 1). An alternative explanation is that native predators' activity is lower near pine plantations because it is a type of habitat very different from the steppe. The low cover of understory vegetation in pine plantations (Paritsis and Aizen 2008) means that small mammals (main pine seed predators in this region) (Nuñez et al. 2008, Chiuffo et al. 2018) have few sheltered habitats to take refuge from predators. Thus, small mammals would reduce risk by spending less time searching for food in the understory of pine plantations than in the adjacent low stature vegetation. The observed seed predation pattern may not reflect *P. ponderosa*'s probability of establishment and invasion because many other factors and processes are involved between seed survival and invasion success (Richardson et al. 2000, Carrillo-Gavilan et al. 2010, Blackburn et al. 2011). However, if seed predators are able to consume most of the seed production of a plant population then its probability of becoming invasive would be highly reduced (Pearson et al. 2012), especially considering further filters before invasion success (Richardson et al. 2000, Blackburn et al. 2011, Carrillo-Gavilan et al. 2012).

Our results also show that the abundance of EMF may not be limiting pine seedling survival and growth at the distance range evaluated. We found a gradient of decreasing root colonization with increasing distance from plantation (Fig. 2). However, this root colonization gradient did not affect seedling survival or growth. Other studies have found important effects of EMF abundance and composition on pine seedling establishment (Nuñez et al. 2009, Hayward et al. 2015, Urcelay et al. 2017) but working with wider distance ranges. For example, Nuñez et al. (2009) found effects of EMF on *P. ponderosa* seedling establishment working with a distance of 1000 m from plantation. Lower levels of EMF root colonization at further distances from the pine plantation may have a significant effect on seedling growth. In this vein, we found that only when seedlings were not colonized (0% EMF root colonization) their growth was negatively affected (Suppl. material 1: Fig. S1). However, if seed survival to predation is limiting at a distance of 25 m from plantation (Fig. 2) the importance of limitations at further distances may not be fundamental to explain invasion in the first hundreds meters from plantations, but may be extremely important for rare but potentially key events of long distance dispersal. Another possibility is that the duration of the bioassay was not long enough to show the effect of root colonization on seedling growth. For example, Nuñez et al. (2009) found effects of *P. ponderosa* root colonization by EMF on seedling growth on a greenhouse experiment that lasted 9 months. However, on a growth chamber the effect of EMF on pine seedling growth should be clear after 6 months, as shown in other studies (Alberton and Kuyper 2009, Hazard et al. 2017). Therefore, our results suggest that EMF availability is not limiting *P. ponderosa* invasion in our study system but that the community of seed predators is behind the observed pattern of low invasion levels for *P. ponderosa*.

Our study shows that biotic resistance can be extremely important in plant invasions, even more important than the propagule pressure and the missed mutualism hypothesis. By contrast, a review of the empirical evidence for general hypothesis in invasion ecology found more support for the propagule pressure hypothesis than for the biotic resistance hypothesis in experiments with terrestrial plants (Jeschke 2014). This highlights the idiosyncratic nature of the importance of these hypotheses. In our study system, only the highest level of propagule pressure overwhelms biotic resistance, highly limiting the invasion of *P. ponderosa*. Therefore, our study highlights the role of biotic resistance in plant invasions.

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Supplementary material 1

Figure S1.

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Sample size for inspection intended to manage risk within mixed consignments

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Abstract

The identification of a lot, and the size of the random sample taken for plant products, is justified by appeal to International Standards for Phytosanitary Measures No. 31, "Methodologies for Sampling of Consignments". ISPM 31 notes that "A lot to be sampled should be a number of units of a single commodity identifiable by its homogeneity [...]" and "Treating multiple commodities as a single lot for convenience may mean that statistical inferences cannot be drawn from the results of the sampling."

However, consignments are frequently heterogeneous, either because the same commodities have multiple sources or because there are several different commodities. The ISPM 31 prescription creates a substantial burden on border inspection because it suggests that heterogeneous populations must be split into homogeneous sub-populations from which separate samples of nominal size must be taken.

We demonstrate that if consignments with known heterogeneity are treated as stratified populations and the random sample of units is allocated proportionally based on the number of units in each stratum, then the nominal sensitivity at the consignment level is achieved if our concern is the level of contamination in the entire consignment taken as a whole. We argue that unknown heterogeneity is no impediment to appropriate statistical inference. We conclude that the international standard is unnecessarily restrictive.

Keywords

ISPM 31, stratification, biosecurity, sample-based regulatory intervention, heterogeneous population

1. Introduction

1.1 Background

Border biosecurity programs are integral to the protection of our natural environments, social amenity, and the economy through prevention of the entry of invasive pests and diseases. The economic cost (either directly, or from control measures) of invasive species has been estimated to be AUD 13.6 billion in Australia (Hoffmann and Broadhurst 2016), up to NZD 3.3 billion in New Zealand (Giera and Bell 2009), CND 34.5 billion in Canada (Colautti et al. 2006) and over USD 200 billion in the United States (Pimentel 2011).

Border inspection for biosecurity is typically the responsibility of national governments and is carried out for verifying the effectiveness of pre-arrival treatments, the detection of material that may pose a biosecurity risk, to gather information about contamination rates, and to deter any potential wrongdoing. Such pre-border and border intervention on a range of imported goods is based on the risk profile of the goods and international agreements.

It is often impractical to inspect all items in a consignment, so only a sample is inspected. In general a consignment would be deemed compliant only if no contaminated units are found in the sample, and non-compliant otherwise. For examples of sampling in the regulatory context, see Robinson (2017) and Venette et al. (2002).

The number required to be sampled is set to provide a certain probability (known as the sensitivity, or confidence level) that at least one contaminated item would be able to be detected from the sample, given a particular prevalence of contaminated items, or less often, given a specified number of contaminated items. The Binomial distribution can be used for large consignments to determine this number.

Formally, the design prevalence is denoted by p, the desired sensitivity by S_d, and the number of units to be inspected by n. The regulator sets the parameters p and S_d, then determines the number of units to be sampled (n), so that the probability that one or more contaminated units is found is greater than S_d. For large consignments we can use the Binomial distribution to obtain the sensitivity

$$S = 1 - (1 - p)^n. \tag{1}$$

Expressing Equation (1) in terms of n gives us the (minimum) number of units to sample to achieve the desired sensitivity S_d, as:

$$n = \log(1 - S_d)/\log(1 - p). \tag{2}$$

As an example, a regulator may set a prevalence (referred to as a design prevalence) at 0.5% and calculate the sample size required to have a 95% chance (the sensitivity) of detecting at least one contaminated item. In this case the required sample is 598, which is almost always rounded to 600 for convenience. Ideally the design prevalence and sensitivity are chosen to provide an acceptable level of residual risk. When the regulator applies this approach, they are accepting that for consignments that do have a prevalence of infested items of 0.5%, in 5% of consignments no contaminated items will be found and these consignments will pass inspection. This example will be used throughout this paper to provide a tangible example of some concepts.

Usually, this sampling occurs within single lines in a consignment; a line comprises a single commodity. Consignments may, however, include multiple lines, either different commodities or the same commodity from different growers. It is natural to assume that identical commodities from different growers might have different levels of contamination. This expectation, combined with the misapprehension that a simple random sample of a consignment with likely heterogeneity would not achieve the desired level of sensitivity, appears to have resulted in the following recommendation under ISPM 31 (International Plant Protection Convention 2008) on the topic of heterogeneous consignments (lots) of plant products:

> *"A lot to be sampled should be a number of units of a single commodity identifiable by its homogeneity in factors such as: origin, grower, packing facility, species, variety, degree of maturity, exporter, area of production, regulated pests and their characteristics, treatment at origin, or type of processing.*
>
> *The criteria used by the NPPO to distinguish lots should be consistently applied for similar consignments.*
>
> *Treating multiple commodities as a single lot for convenience may mean that statistical inferences cannot be drawn from the results of the sampling."*

This prescription implies that in order for a heterogeneous consignment to satisfy the regulatory biosecurity requirements based on achieving a desired level of sensitivity (e.g. 95%) and a given design prevalence (e.g. 0.5%), it must be split into its homogeneous lines, and these must each be subjected to, for example, the 600 unit sample.

In what follows we consider that the contamination rate of the consignment as a whole is equal to the design prevalence, accepting that the rate within different parts of the consignment might be higher or lower than this value, and show that if the sample is split proportionately between the different parts, the sensitivity is at least as high as the value derived based on a single homogeneous consignment.

1.2 This paper

The goal of this paper is to demonstrate that ISPM 31's recommendation against mixing heterogeneous lines (lots) is unnecessarily restrictive, and that there are ways of sampling mixed lines that do achieve the required sensitivity against contamination without increasing the number of units we need to include in the sample.

Some critical assumptions are still required. First, we assume that the regulator is happy to apply their compliance rule to the entire consignment. In other words the entire consignment will only be deemed compliant if the sample taken from the consignment returns no contaminated items. Under this assumption the regulator is not specifically worried about higher levels of contamination in some lines, as long as the overall contamination rate of the consignment satisfies their design target. However, under this approach, if contamination is detected in any of the units sampled, then all of the lines from the consignment must be rejected. Second, our solution involves treating the lines in the consignment as if they were strata. We assume that once the sample is split, the required number of units from each line are randomly selected from the respective lines.

We show that the act of stratifying the consignment by line and then allocating the total inspection sample (e.g. the 600 unit sample) proportionally to the stratum population counts will deliver nominal sensitivity (at least 95%) against a given overall contamination rate (0.5% as an example). Jointly, these arguments suggest that ISPM 31 is currently too restrictive in its prescription for mixed consignments.

2. ISPM 31 and heterogeneity

The sole statistical reference provided for the ISPM 31 sample size calculations is Cochran's 1977 Sampling Techniques (Cochran 1977), and the calculations themselves can be located within a body of work called "design-based sampling theory". Importantly, there is no statistical constraint or requirement for homogeneity of a sampled population within design-based sampling theory (Cochran 1977). Indeed, samples are commonly collected and analyzed across substantially heterogeneous populations, such as human and economic populations in official statistics, and forest communities in natural resource management. The only constraints are (i) that the sample be taken according to one of a number of different kinds of random sample designs, for example as detailed in ISPM 31, and (ii) if contamination is detected in any of the units sampled, then all of the lines from which samples were taken must be rejected. If the heterogeneity is unknown within a single diverse line then a simple random sample will deliver nominal sensitivity by design.

2.1 Dividing our sample between multiple lines

We now consider in detail sampling from multiple lines within a consignment. Suppose that the regulator believes it to be appropriate to sample across the K lines of a consignment as though they were a single mixed line. While we accept that each line might have a different prevalence, our criterion is that the overall prevalence in the consignment is equal to the design prevalence.

We shall find which combination of line prevalences (that satisfy the design prevalence) corresponds to the smallest overall sensitivity. By basing our calculation of the total number n of samples required on that combination of prevalences, we will ensure that the sensitivity of the inspection will be always greater than the required design sensitivity, S_d.

We shall sample a proportion w_k of the total sample from line k. Hence the sample size per line is $n_k = w_k n$, such that $\sum_k w_k = 1$. There are N_k units in the k^{th} line making a total of $\sum_k N_k$ units.

If there are d_k contaminated items in line k we could use the Hypergeometric distribution to calculate the probability that none of these would be found. The result is mathematically intractable, and it is both more convenient and more conservative in regulatory contexts to use the Binomial approximation[1] based on a contamination rate expressed as a proportion of $p_k = d_k / N_k$. The joint contamination rate, p (our design prevalence), satisfies $\sum_k N_k p_k = N.p = \sum_k d_k$.

When sampling from multiple lines, the sensitivity of the inspection is of the same form as Equation (1), namely

$$S = 1 - \prod_{k=1}^{K} (1 - p_k)^{nw_k}. \tag{3}$$

Minimizing Equation (3) is equivalent to maximizing $\sum_k nw_k \log(1 - p_k)$, subject to the constraint placed by the joint contamination rate, $\sum_k N_k p_k = N.p$. It is straightforward to show by the method of Lagrange Multipliers (Lagrange 1811) that the combination of p_k for which the sensitivity is least is:

$$1 - p_k = (1 - p) w_k \frac{N}{N_k}. \tag{4}$$

We will now consider the optimal values for the weights w_k, beginning with the best choice, which is splitting the sample proportional to the line sizes.

2.2 Dividing the sample size proportional to the line sizes

In this section we set the sample size for each line proportional to the line size, that is $w_k = N_k / N$. Substituting these values into Equation (4), we find that the sensitivity will be minimized when $p_k = p$. Substituting these values of p_k and w_k into Equation (3), shows that the required sample size is identical to Equation (2). This choice of n and weights $w_k = N_k / N$ ensure that the realised sensitivity will be no worse than the design sensitivity, irrespective of the individual line prevalences that satisfy the design prevalence.

The total sample size is the same as if we were sampling from a homogeneous population, as evidenced by the finding that having the same prevalence in each line corresponds to the combination of prevalences that gives the minimum sensitivity if

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[1] We note that calculations based on the Hypergeometric distribution are appropriate for very small consignment sizes and/or when the inspection method is destructive and the number of samples taken needs to be minimized. In this situation it will most likely be the case that interest lies in sampling from a single line, not multiple lines as assumed in this manuscript.

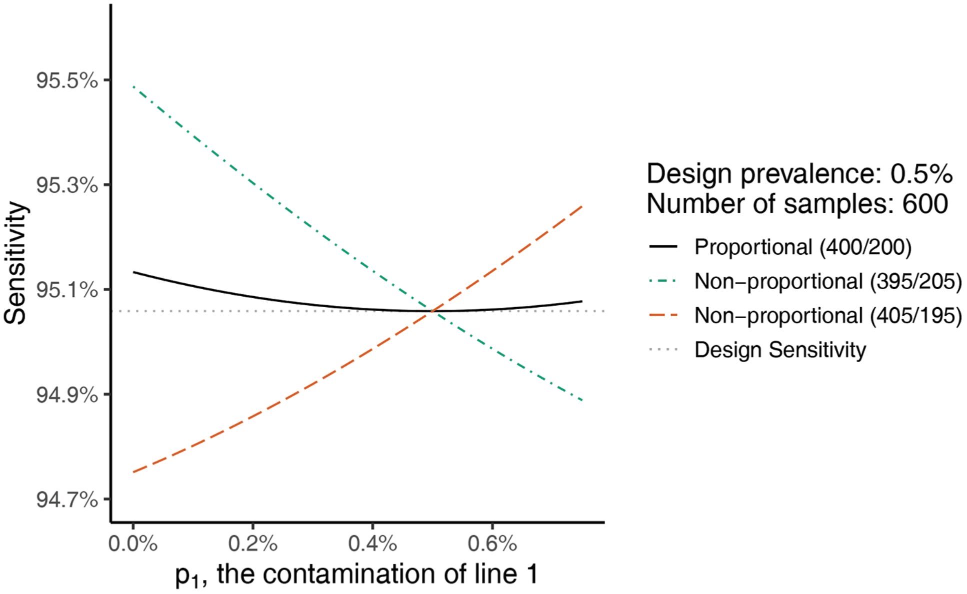

Figure 1. Achieved sensitivity obtained from different allocations of the 600 units when the prevalence in each line varies so that the overall prevalence is 0.5%. The solid black line corresponding to a proportional split is always greater than the desired sensitivity. For non-proportional allocation, the sensitivity is sometimes greater and sometimes less than desired.

we choose our weightings to be proportional to the line size. For any other combination of line prevalences that overall meet our design prevalence, the sensitivity of the inspection will be greater than the design sensitivity.

Figure 1 compares proportional and non-proportional allocation by way of an example; a consignment with two lines where one line has 20000 units and the other has 10000. We wish to find contamination present at the design prevalence of 0.5%, with 95% sensitivity. As already mentioned this requires a 600 unit sample (which actually corresponds to a 95.06% sensitivity). Consider three allocation schemes: the proportional allocation as just derived, requiring a sample of 400 units from the first line and 200 units from the second, and two non-proportional schemes where the sample sizes in each line are 395/205 units and 405/195 units respectively.

Figure 1 demonstrates the achieved sensitivity that would result from each allocation scheme as a function of the true contamination rate of the first line. The solid line shows the achieved sensitivity if we used proportional allocation, the horizontal line shows the nominal sensitivity, and the other lines show the two sensitivities achieved by the non-proportional allocation schemes. The key feature to note in Figure 1 is that the achieved sensitivity is *always* greater than the nominal sensitivity of 95% under proportional allocation, whereas it may be less under non-proportional allocations for some prevalence combinations that meet the design prevalence.

Figure 2 provides a similar comparison for a consignment of three lines for which the prevalences in the lines vary such that the overall prevalence is 0.5%. The figure

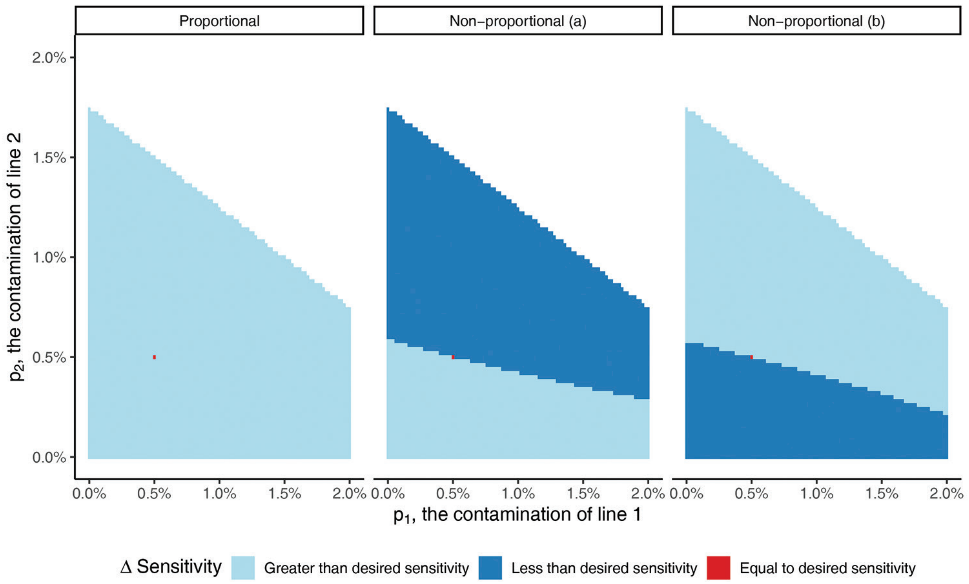

Figure 2. Difference in achieved sensitivity under three different sampling situations. The values plotted show the regions of obtained sensitivities that are greater than or less than the desired sensitivity.

shows those prevalence combinations for which the sensitivity would be less (or greater) than that desired. The left hand panel shows that the obtained sensitivity is never less than the desired sensitivity under proportional allocation. The middle and right panels are for different non-proportional division of the sample numbers: both show that there are values for which the obtained sensitivity is less than desired.

2.3 Variations of the problem

There are a number of minor variations to the problem of splitting the sample size between a number of lines. The derivations are not given but follow a similar method to the above.

2.3.1 Imperfect inspection

Sometimes our inspection will not be fully effective, and we have a probability e_k that inspection of a contaminated item in line k will detect the contamination. When our inspection method is less than perfect, we need to take more samples to compensate. It is convenient to define $M_k = N_k/e_k$ and $M = \sum_k M_k$. If we divide our sample between lines according to the fraction M_k/M (rather than N_k/N), we can show that the minimum sensitivity occurs when the *apparent* prevalence $(p_k e_k)$ in each line is the same by using the method in Section 2.1. From that we find that the number of samples required should be based on an adjusted (smaller) prevalence $q = Np/\sum_k M_k$ to give $n = \log(1-S_d)/\log(1-q)$ and $n_k = nM_k/M$.

2.3.2 Design prevalence as an absolute number

Occasionally the design prevalence is specified as an absolute number D of contaminated items. Replacing p by D/N in the above gives the required sample size which, as before, would be split proportionally between the lines:

$$n = \frac{\log(1 - S_d)}{\log(1 - \frac{D}{N})}$$

For an absolute design prevalence, $\log(1-D/N)$ needs to be calculated for each consignment. To simplify this, one can increase the sample size slightly by using the approximation $\log(1-D/N) \approx -D/N$ (which is equivalent to using the Poisson approximation to the Binomial). The fraction

$$\frac{-\log(1 - S_d)}{D}$$

can be agreed upon by the regulator and pre-computed. This gives the overall number sampled being proportional to the number in the consignment:

$$n = \frac{-\log(1 - S_d)}{D} N.$$

2.3.3 Not knowing line sizes accurately

So far we have assumed that the counts for each line are accurately known. If the percentage errors in the counts are likely to be similar, this will be of little concern, since the relative contribution each line makes to the total will stay much the same. If, however, there is more uncertainty, the number of samples required needs to be increased for each line.

Suppose that we think the actual line sizes could be between $N_k(1-\alpha_k)$ and $N_k(1+\beta_k)$. The consignment size would be between $N(1 - \alpha)$ and $N(1 + \beta)$, the sum of the lower and upper line sizes respectively. Hence the weighting for line k should lie between

$$\frac{N_k(1-\alpha_k)}{N(1+\beta)} \text{ and } \frac{N_k(1+\beta_k)}{N(1-\alpha)}.$$

To be conservative, we use the upper limit of this range to determine the number of samples per line in terms of calculated based on Equation (2) using our desired sensitivity and design prevalence:

$$n_k = n\frac{N_k(1 + \beta_k)}{N(1 - \alpha)}.$$

Our uncertainty about line size means that we need to take more samples in total, namely

$$n\frac{1+\beta}{1-\alpha} \approx n(1+\alpha+\beta).$$

As an example, if our uncertainty of the size of the consignment was of the order of ±10%, then we need to increase the sample size by approximately 20%.

2.3.4 Using fixed sample sizes

Regulators might wish to choose fixed sample sizes for each line, rather than allocate sample sizes proportional to the line sizes. For example, we could take an equal number of samples from each line. However, for such weightings, more samples are required in order to ensure the design sensitivity S_d is met. For all practical purposes, the number of samples (*m*) required for fixed sample sizes has to be chosen so that for each line the number of samples taken, say $m_k = w_k m$, is greater than or equal to

$$n_k = n\frac{N_k}{N},$$

the number of samples required if proportional weightings had been used.

3. Discussion and conclusions

We have shown how a standard sample size may be split between a mixed-line consignment using proportional allocation, while still at a minimum giving the desired chance of detecting contamination if it is present at a specified rate for the entire consignment. Of course, a truly random sample from the entire consignment will also give the desired sensitivity regardless of any clustering of contamination in the consignment and on average would result in a proportional number of samples being taken from each line. However, the latter approach by chance could result in no or very few samples being taken from lines with small numbers of items, something regulators might be uncomfortable with. Adopting proportional allocation would provide an explicit starting point from which samples in such lines could be increased.

If this approach to sampling is employed, it is critical for exporters to understand that if contamination is found in just one line, the entire consignment has not satisfied the import requirements and would be deemed to have failed the inspection with the resultant consequences.

The reverse is true for regulators: it is important that they do not deem only the lines in which contamination was found as non-compliant and accept the rest. The lines in which no contamination has been found have not had sufficient inspection to demonstrate that they meet the design sensitivity and prevalence requirements. Further, simply taking more samples from the 'clean' lines to 'top up' the sample size to e.g. 600 units from those lines is not enough. The actual calculation of sample sizes for such 'topping-up' is outside the scope of this paper. Suffice to say that the initial sample size for such a scheme must be greater than 600 units because, as well as the possibility of incorrectly accepting the consignment after the first sample, the regulator might incorrectly accept the remaining part of the consignment after the second sample.

We note that there are reasons for which processing lines separately makes operational sense. For example, the products may carry different kinds of pests that themselves present different risks, may have different levels of detection probabilities, and even different treatment possibilities. Another reason is that the exporter may not wish to take the chance that contamination in one line will affect the treatment of all of the lines in the consignment.

Our result relies on the assumption of exact proportional allocation of the samples to lines based on their counts. In some situations, the number of units in a line might differ from the nominal count, so that an exact proportional allocation would not be made. We have shown that increasing the sample size in proportion to the likely variation provides a way to ensure that the desired sensitivity is still met.

Furthermore, our result assumes that the sampling is done randomly within each line. If contamination is likely to be clustered and the sampling is not random (for example inspecting all fruit within a number of randomly-selected boxes) a different method must be used to determine the sample size (e.g. Venette et al. 2002). Extending such results from a single line is outside the scope of this paper.

Using a proportional allocation of the sample might not be prudent when the number of items in one line greatly exceeds the number in the other lines. An example of this might be with one line being melons, and one of the other lines being cherries. The problem is that proportional allocation might result in only one or two units being selected from lines with few units. While the lines with few units might only contribute a small proportion of the contamination, there may be misgivings that they haven't been adequately inspected. One way this could be resolved is by considering them to be, from the point of view of sampling, two separate consignments. Another alternative might be to consider a box of cherries as the unit, which might give comparable unit numbers in the lines.

Another solution might be to top up the calculated number of samples to make a minimum sample per line. This would guard against missing gross contamination in a line with few units which, while not contributing greatly to the overall contamination, would be of concern if present. For example, a minimum sample of 30 in a line would detect a contamination rate of 10% in that line with a 95% probability. The other advantage in having a minimum sample size would be that information about that particular item type or source would be more quickly accumulated.

If the types of contamination in some lines are thought to have greater consequences than others, one could take extra samples above what is required in those lines, for example take twice as many. While taking extra samples is a form of non-proportional allocation, it is based on the number determined by proportional allocation: taking extra samples above the proportional allocation would increase the sensitivity of the inspection. However, to ensure the design sensitivity is met for a more general division of the sample numbers between lines (such as equally between the lines), no line should have fewer samples taken from it than the number determined by proportional allocation.

Finally, it cannot be emphasized enough: when the sample is stratified proportional to the stratum size, if contamination is found, even if it is in just one line, the whole consignment has to be deemed non-compliant and subject to whatever requirement non-compliance imposes. If this is not acceptable, then individual lines (or groups of lines) must be inspected separately, with each component subject to the specified compliance test.

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