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DOI: 10.1017/inp.2024.24

Short title: Regulating cultivars & hybrids

Regulatory options for cultivars and hybrids of invasive plant species – the South African experience.

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Abstract

Invasive plant taxa are generally regulated at the species level, without considering infra- or inter-specific variation. However, cultivars or hybrids can be of a lower level of risk, e.g., due to sterility. We evaluate six general approaches to regulating cultivars and hybrids - 1) “Globally guilty by association”; 2) “Nationally guilty by association”; 3) “Guilty until proven innocent”; 4) “Negotiated guilt”; 5) “Claimed to be innocent”; and 6) “Innocent until proven guilty”. We discuss these approaches in the context of South Africa (which has a typified “Negotiated guilt” approach). Following negotiations since 2001 between the South African horticultural industry/green industry and legislators, an unofficial consensus list of “presumed sterile” cultivars and hybrids was produced in 2014 containing 187 entities from 34 taxa. In 2020 this was reduced to 157 entities from 16 taxa. But the evidence supporting the original lists and the subsequent revisions were not published. To address this issue, we developed a generic pro-forma for reporting sterility based on observations and/or experiments on: flowering, fruiting, pollen, and seeds; the potential for vegetation propagation; and the potential for genetic changes (including hybridisation and reversion to fertility). We recommend that such information should be incorporated into risk analyses conducted specifically for infra- and inter- specific entities, and only if the risk of a harmful invasion is demonstrated to be acceptably low or can be easily mitigated should such entities be exempted from regulation. This will be time-consuming, but by setting out the evidence clearly, the approach is transparent and provides a clear route for stakeholders to seek exemptions for entities of importance. In summary, although we suspect the simplicity of the “Negotiated guilt” approach is desirable to many stakeholders; in general, and specifically for South Africa, we recommend a shift towards the “Guilty until proven innocent” approach.

Keywords: invasive taxa, sterile cultivars and hybrids, regulatory approaches, stakeholder negotiations, regulatory lists

Management Implications

We outline six approaches to regulating cultivars and/or hybrids of invasive plant species. Each approach requires different levels of resources and evidence and results in different levels of risk. We outline the consequences of the different approaches so regulators and stakeholders can choose the best option for their needs.

In general, we recommend a "Guilty until proven innocent" approach. All cultivars or hybrids that are related to at least one regulated taxon should be similarly regulated unless and until there is documented evidence that the cultivar/hybrid is not invasive (i.e., risk analyses should be conducted for each exempted entity). This approach aims to restrict harmful invasions but also provide stakeholders with a process to motivate for the exemption of entities that are valuable to them (albeit one that requires substantial evidence for a change to be made). We provide a pro-forma to support reporting low levels of invasiveness based on observations and/or experiments. We also note that, to be effective, the approach also requires that the public (and nursery customers) are aware of the process and that sterile cultivars/hybrids can be easily distinguished in practice.

In South Africa, exemptions have been made for several cultivars and hybrids on the basis of presumed sterility. These exemptions emerged from a series of lengthy negotiations between the South African horticulture industry and the regulators and can be typified as a "Negotiated guilt" approach. This approach requires less resources and is much simpler to execute, but likely leads to more entities being considered safe which are in fact harmful. We motivate for a shift to the "Guilty until proven innocent" approach.

Introduction

Biological invasions have negative impacts on ecosystems and economies (Pyšek et al. 2012; Vilà et al. 2011; IPBES 2023). To combat or prevent these negative effects various regulations at different scales (local, regional or national) have been developed (Hulme et al. 2018; Turbelin et al. 2017), often including the use of regulatory species lists (García-de-Lomas and Vilà 2015; Pergl et al. 2016). These regulatory lists usually focus on taxa that are known or perceived to be harmful (cf. Kumschick et al. 2024 for a discussion on lists of taxa that are of low risk), though all such lists can be complex to develop and will have uncertainties (McGeoch et al. 2012). One particular issue is that non-native plant taxa are assessed for their invasion risk at the species level (Kumschick and Richardson 2013) and thus are also regulated at this level, i.e., species are listed, with minor attention given to infraspecifc (such as cultivars, forms or varieties) or interspecific (hybrids) entities [e.g., in Poland (Tokarska-Guzik et al. 2021) and Japan (Mizutani and Goka 2010)].

At the population level, many plant species are individually clustered into distinct genetic lineages, across their geographical ranges, suggesting an adaptation to local conditions (Linhart and Grant 1996; Leimu and Fischer 2008; Hereford 2009). As such, Gotelii and Stanton- Geddes (2015) suggest that infraspecific variation needs to be considered when modelling shifts in the geographical ranges of plant populations. Thus, there can be large variation amongst infra- and inter- specific entities, and the parent species. For example, infra-specific entities of *Acacia saligna* occupy different bioclimatic niches within the species native range, suggesting that this could also be the case in its invasive range (Thompson et al. 2011). Infra-specific variation can also translate to differences in the impacts caused by invasive taxa, such as spineless cultivars of *Opuntia ficus-indica* which are presumed to be non-invasive due to increased herbivory that regulates the population (Zimmermann and Granata 2002, Novoa et al. 2018).

Hybridisation in plants can occur between (interspecific) or within (infraspecific) species resulting in several possible genetic changes (Landry et al. 2007) which can increase or reduce fitness (Charlesworth and Willis 2009). Such genetic changes can influence invasion success (Buhk and Thielsch 2015), and there are many examples of invasive taxa that evolved after inter-taxon hybridisation (Ellstrand and Schierenbeck, 2000; Hovick and Whitney, 2014; Dlugosch et al. 2015). Infraspecifc hybridisation can also promote invasiveness, as seen in the case of *Pyrus calleryana* (Culley and Hardiman 2009).

https://doi.org/10.1017/inp.2024.24 Published online by Cambridge University Press

Conversely, hybridisation has been used a tool to develop sterile hybrids of known invasive taxa, such as the "presumed sterile" hybrids of *Ruellia simplex* which were fruitless with low pollen viability (Freyre et al. 2012).

It is evident that infra- and inter-specific entities can either pose a lower or higher invasion risk. Therefore, not considering infra- or inter- specific variation when developing invasive species policies, could lead to inaccurate invasion risk estimates of such taxa (Gordon 2016). Economic losses arising from regulating taxa with high ornamental values can cause conflicts of interests amongst stakeholders, industries, and regulators (Wirth et al, 2004). As such, there is often a demand to develop, and exempt, infra- or inter-specific taxa that are "safe" or "non-invasive" (Guo et al. 2004; Freyre et al. 2014).

There have been various attempts to develop sterile cultivars (e.g., Brand et al. 2012; Spies and du Plessis 1987; Wilson and Mecca 2003; reviewed by Datta et al. 2020), via methods such as genetic modification (Kanaya, 2008; Mitsuda et al., 2006), inducing polyploidy (Thammina et al. 2011) or interploid hybridisation (Czarzencki et al. 2012; Deng et al. 2020). Czarnecki et al. (2012) and Deng et al. (2020) successfully bred and recommended various sterile cultivars of *Lantana camara* for ornamental use based on their relatively low seed production and viability. Other examples of sterile cultivars of invasive plants include *Spiraea japonica* (Wilson and Hoch 2009), *Acer platanoides* (Conklin and Selmer, 2009) and *Nandina domestica* (Knox and Wilson 2006). Even though these taxa were considered safe and non-invasive, this might change with plant age, since "presumed sterile" cultivars of Japanese Barberry (*Berberis thunbergii*) for example, that were initially seedless started to produce seeds when plants were much older (Brand et al. 2012). Further, cultivars that have significantly lower seed germination and viability percentages need not necessarily have lower population growth rates (Knight et al., 2011). Therefore, it is crucial that sterility is comprehensively assessed before deeming any infra- or inter-specific entity as sterile.

Frameworks and protocols have been developed to identify safe/non-invasive cultivars of invasive plant species. Datta et al. (2020) framed a set of six questions that must be answered before a cultivar or hybrid is deemed safe. These six questions incorporate the main components of a risk analysis (risk identification, risk assessment, risk management, and risk communication) (Kumschick et al. 2020a, 2020b). Another example is the Infraspecifc Taxon Protocol (ITP), a science-based assessment tool developed by researchers in Florida, USA, to assess cultivars with the potential of reduced invasiveness (IFAS 2008;

Knox 2008). This tool contains a series of questions of which the responses gather evidence that the cultivar: 1) can be easily distinguished from the wild type species, 2) has traits that could reduce dispersal and/or spread, 3) is incapable of hybridising with native flora, 4) does not readily revert to natural or invasive form, and 5) is likely to have a lowered ecological impact. Applying the ITP protocol, the cultivars of Heavenly Bamboo *Nandina domestica* "Fire Power" (Knox and Wilson 2006; Knox 2008) and "Harbour Dwarf" (Knox and Wilson 2006) were deemed safe for Florida. Tools such as the Datta et al. (2020) framework and the ITP protocol can assist with developing regulations for infra and inter- specific entities.

Very few regions have considered infra- or inter- specific entities for invasive taxa regulations. In the United States of America (USA), many states adopt independent procedures and protocols to identify and assess the impact of invasive species (Lakoba et al. 2020; Beaury et al. 2021). In Florida, as discussed previously the ITP protocol is used. In Oregon, sterile cultivars can be approved for statewide sale if their seed production is less than 2%, however, a fee must be paid to Oregon State University to conduct a study which evaluates the fecundity of a specific taxon (Culley et al. 2016), thereafter the results must be submitted to the Oregon Department of Agriculture for verification. In Minnesota and Wisconsin, decisions on cultivar bans or acceptability are based on scientific data pertaining to specific cultivars (Brand et al. 2012). Such approaches are backed up by scientific evidence, providing more confidence in the regulatory decisions, and thus results increased research conducted to acquire evidence for safe/non-invasive cultivars within a specific region (Wilson and Deng, 2023). Other states adopt different approaches, such as a decision-making tree to underpin cultivar exemption in New York [(The New York State Code of Rules and Regulations (NYCRR) Part 575, 2014] and the formation of a committee to explore sterile cultivar exemptions in Massachusetts, all of which are still evidence-based (Brand 2016).

These examples show that the level of sterility deemed acceptable, and in fact how sterility is defined, varies. For example, anecdotal evidence from the South African horticulture industry suggests that the term sterile was used to define a plant that is unable to escape from cultivation, and so still potentially able to produce viable seeds, therefore, we use the term "presumed sterile" here (see Table 1). The National Environmental Management: Biodiversity Act, Alien & Invasive Species (NEM:BA A&IS) regulations (Department of Environmental Affairs 2020), attempt to accommodate infra- and inter-specific variation in plant taxa, by granting exemptions for cultivars or hybrids based on sterility. However, the evidence for exempting these "presumed sterile" cultivars and hybrids has not been published (Wilson & Kumschick 2024).

In this article we first examine different approaches to regulate cultivars and hybrids of invasive plant species, secondly, we review how cultivars and hybrids of invasive plant species could be regulated by using South Africa as a case study, and lastly provide guidelines for assessing sterility in cultivars/hybrids of invasive plants species.

Approaches for regulating cultivars and hybrids of invasive plant species

For the regulation and subsequent management of invasive species, Kumschick et al. (2012) suggest that a transparent process is needed, which clearly expresses all the options by identifying and discussing the pros and cons of each. We present six approaches to regulate cultivars and hybrids of invasive plant species viz. (1) "Globally guilty by association"; (2) "Nationally guilty by association"; (3) "Guilty until proven innocent"; (4) "Negotiated guilt"; (5) "Claimed to be innocent", (6) "Innocent until proven guilty". The rationale, predicted number of entities banned, evidence required, expected number of listing errors, and ease of implementation are shown for each approach in Table 2.

The rationale and process required for the regulatory approaches

The approaches range from risk-adverse ("Globally guilty by association") to reactive ("Innocent until proven guilty"). As such, the approaches differ in the degree to which extrapolations of risk are made, resulting in a different risk assessment process for each approach. For the first two approaches "invasive elsewhere" is the only type of evidence required to ban cultivars or hybrids of an invasive taxon, thus no formal risk assessment process is required, rather these approaches are based on the precautionary principle and will have its own implications (see Table 2). Approaches 3 ("Guilty until proven innocent") and 4 ("Negotiated guilt") although evidence-based approaches, still initially ban all cultivars and hybrids of the regulated invasive taxa, however, cultivars and hybrids of the regulated invasive taxa are allowed to be exempted from regulations if the required evidence is made available. The third approach requires scientific experiments to be conducted to demonstrate the safety of the cultivar or hybrid (this demonstration is usually in terms of sterility, see discussion on this later) which should feed into a formal risk analysis process for the exemption to be recommended. The fourth approach, also evidence-based, does not require experiments to be conducted to demonstrate non-invasiveness, instead, the level of 'guilt' - or rather invasive potential of a cultivar - is negotiated between the regulators and stakeholders

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(i.e., a formal risk analysis may not be required) (Table 2). The fifth approach ("Claimed to be innocent") requires no evidence, and cultivars or hybrids can be exempted if any stakeholders claims that the entity is non-invasive (see Table 2 for the implications of such an approach). The last approach ("Innocent until proven guilty"), also does not require any evidence for safety. No formal risk assessment or risk analysis is required, all cultivars and hybrids of the regulated invasive taxa are exempted, unless there is specific evidence of invasiveness of the cultivar or hybrid, i.e. it can be seen as a reactive approach (Table 2).

The implications of the various regulatory approaches

Each approach has different implications as outlined in Table 2, and differ in how easy they are to implement. The most resource intensive approaches are those that require specific risk analyses to be conducted (Approaches 3-4, Table 2). If cultivars or hybrids are granted exemptions, it is important that those entities can easily be distinguished from their parental genotypes in practice, thus avoiding confusion when such exemptions are implemented. It is also important to note that each approach would vary in other factors such as number of entities banned, the effort required to demonstrate safety and the expected number of listing errors (as outlined in Table 2), all of which are important aspects to consider when selecting a regulatory approach to implement.

Given invasiveness and impacts have some phylogenetic signal (Diez et al. 2012) we suggest that a reactive approach would be extremely risky, especially for cultivars or hybrids that have known invasive parent genotypes or congeners. On the other end of the spectrum adopting the "Globally guilty by association" or "Nationally guilty by association" approach would likely mean a high number of infra- and inter- specific taxa were unnecessarily banned, as risk assessments are not done at the infra- or inter- specific taxonomic level (Gordon, 2016). Such approaches could lead to significant economic losses in the green industry, potentially generating substantial disputes between the green industry and regulators, particularly if there was no clear route to contest the listing of taxa that stakeholders perceive to be safe.

Regulation of "presumed sterile" cultivars and hybrids in South Africa

In the following section, used South Africa as a case study to review the regulation of "presumed sterile" cultivars and hybrids of invasive taxa. There have been various negotiations between the South African horticultural industry and the Department of Forestry, Fisheries and the Environment (DFFE) of South Africa regarding the A&IS Regulations and "presumed sterile" cultivars and hybrids (pers. com Kay Montgomery).

Since all of the "presumed sterile" (discussed below) cultivars or hybrids registered in South Africa (see Supplementary Table S1) are important ornamental plants (see Figure 1 A-D for examples) or horticultural trees (Armitage, 2008; www.gardeninginsouthafrica.co.za, accessed 30 May 2023), the horticulture industry is a crucially interested and affected stakeholder. Thus, in 2001 the South African horticulture industry (Figure 2) initiated the negotiations with the government (Figure 2) to prevent the regulation of taxa that were horticulturally and economically significant. The negotiations entailed discussions regarding the invasiveness (or lack thereof) of those taxa prior to implementation of the regulations.

In 2004, the National Environmental Management: Biodiversity Act (NEM:BA) (Act No. 10 of 2004) was enacted (see Lukey and Hall, 2020 and Wilson and Kumschick, 2024, for a detailed review of the history of invasive species regulations in South Africa), and as part of the act, the Alien and Invasive Species (A&IS) Regulations were promulgated in 2014 (Department of Environmental Affairs, 2014). Under revised lists of October 2020 (Department of Environmental Affairs, 2020), 382 plant taxa were listed (Wilson 2024). In 2004, when NE:MBA was enacted, the South African horticulture industry requested exemptions for cultivars and hybrids for specific taxa, as such, the NEM:BA A&IS Regulations address infra- and inter- specific variation by granting exemptions for cultivars or hybrids based on their presumed sterility.

As such, the negotiations moved towards exemptions of cultivars and hybrids that were seemingly safe. These negotiations were lengthy and in 2010 (Figure 2), it was decided to implement the "polluters pay" principle suggesting that those responsible for causing harm to the environment should be responsible for the cost of such damage (Luppi et al. 2012), increasing conflict between the negotiating parties. It was agreed that a consensus had to be reached, but there was still a lack of scientific evidence regarding the invasiveness of the conflict taxa.

Eventually, a consensus was reached in 2014, and as part of this consensus, prior to the first NE:MBA A&IS list in 2014 (Department of Environmental Affairs, 2014), the DFFE gave the industry an opportunity to propose a list of those cultivars and hybrids which it regarded to be sterile (Figure 2; the unofficial consensus list; see Supplementary Table S1). As such, one of the major outcomes of the negotiations was the unofficial consensus to

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list/register "presumed sterile" cultivars and hybrids proposed by the South African horticulture industry in 2014. This list contained 187 cultivars and/or hybrids for 34 taxa (see Supplementary Table S1). This "presumed sterile" taxa list has changed over time (Figure 2) and currently contains 157 "presumed sterile" cultivars and/or hybrids from 16 taxa (Table 3; Supplementary Table S2). However, the evidence for the sterility of the registered cultivars and hybrids is lacking. Rather, the argument for the requested exemptions was on the basis that none of the cultivars nor hybrids had recorded naturalised populations. For legislative purposes, the government termed these "presumed sterile" taxa as sterile cultivars and hybrids, bringing forth the inception of the term sterile cultivars and hybrids in the South African context (see Table 1). However, the industry and DFFE did not publish evidence of sterility for "presumed sterile" cultivars and hybrids (see Table 4 for anecdotes from the green industry regarding "presumed sterile" cultivars). The first iteration of the list of taxa formally listed with "presumed sterile" cultivars and hybrids contained 34 taxa (Supplementary Table S1; Figure 2).

In 2017 revisions to the unofficial consultative list included the removal of *Coreopsis lanceolata* cultivars due to a lack of evidence of sterility, and the removal of *Vinca major* cultivars due to the plant spreading more vegetatively than sexually (Supplementary Appendix; Figure 2). The industry was given deadlines (one to two years) to prove the sterility of various listed cultivars and hybrids if they were to remain on the unofficial consultative list (Supplementary Appendix). In the 2020 iteration of the NE:MBA A&IS Lists (Department of Environmental Affairs, 2020), only 16 taxa (previously 34) had regulatory provisions for exemptions of "presumed sterile" cultivars (Figure 2; Table 3, Supplementary Table S2). However, we are not aware of publicly available evidence of sterility for the retained entities nor of the precise reason why specific entities were removed from the lists. Thus, it is important to understand how the list proposed by South African horticulture industry was developed so a transparent, evidence-based approach can be used in future for producing lists that guide policy and action (Butchart et al. 2010; Perry and Perry 2008).

[Insert **Figure 2**].

What should a sterility assessment for cultivars or hybrids of invasive species include?

Here, we outline the basic requirements for sterility assessments of cultivars/hybrids of invasive plant species and present a generic pro-forma for reporting on sterility (Figure 3).

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It is important to note that the guidelines presented are not a standardised protocol for sterility assessments but aim to outline various components that should be included in any sterility assessments for invasive plants and provide examples of the types of experiments that could be conducted to gather the required data. The first three components of the sterility assessment specifically deal with assessing the sexual reproductive pathway, the fourth with quantifying asexual reproduction and the last component assesses the stability of sterility.

1 – Flower and fruit production:

Do the cultivars/hybrids produce flowers and fruit, and if so, how many? Common-garden or greenhouse experiments (e.g. Knox and Wilson, 2003) can be set up by growing replicates of each tested cultivar/hybrid for a period of time (until reproductive maturity). Wild type plants can be grown as controls.

2 - Pollen analyses:

If the tested cultivars/hybrids produce flowers, pollen viability analyses should be done. Pollen viability assessments are often done using biological staining techniques (Jones, 2012; Pinillos and Cuevas, 2008) or by conducting pollen germination experiments. For the control of these experiments, pollen from wild type plants (representing the invasive forms) of the tested cultivar/hybrids should be used, and the pollen viability and/or germination percentages should first be significantly lower in tested hybrids/cultivars than in wild type. Ideally, no flowers (i.e. no pollen produced) should be recommended as a truly sterile cultivar/hybrid. However, this component of sterility/fertility can be defined if pollen viability/germination is below a certain percentage threshold. It should be up to the legislators and stakeholders within a region/country to agree on an acceptable threshold as this can vary between various taxa depending on other factors such as time to reproduction (different in fast and slow growing species), benefits of cultivar (environmental, economic, or social) and results from seed analyses (step 3). Finally, electron microscopy can be used to supplement pollen viability results which may identify abnormal pollen grains characteristic of low pollen viability (Shaik et al. 2022).

3 - Seed analyses:

If cultivars/hybrids produce fruit (and subsequently set seed), the number of seeds per fruit should be quantified. Thereafter, seed viability and germination assays (e.g. Czarnecki et al., 2017; Deng et al., 2020) should be conducted. Seed viability assays can be done using the

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standard tetrazolium test and germination assays can be conducted *in vitro* (petri dishes and incubators) *or ex* vitro (seed sowing in soil). Ideally, for this component, a truly sterile cultivar/hybrid would not produce any seeds or should have seeds that have 0% viability or germination. However, as per the previous step, an acceptable threshold can be defined, which may be context specific. Various other/additional seed analyses can be done (seed mass/ultrastructure analyses, etc) to understand the mechanisms of sterility.

These three components provide insights into the degree of fertility of a cultivar or hybrid. The next two steps aim to provide a more comprehensive understanding of the invasion risk.

4 – Potential for vegetative propagation:

Can the cultivars/hybrids reproduce asexually? Cuttings propagation (or other types of vegetative propagation, such as bulbils) experiments can be conducted to determine the survival/success rate of cuttings to gain an understanding of how easily the plants can propagate (or spread) asexually. Further, it is advised that basic vigour assessments be conducted with the surviving cuttings to determine which are the fastest growing cultivars/hybrids (a trait that increases invasive potential) .

5- Potential for genetic changes:

Lastly, to determine the potential for genetic changes which could affect the stability of sterility cross breeding between cultivars and between cultivars and wild type plants should be investigated. Hand-pollination experiments in the greenhouse are recommended (e.g. Wilson and Hoch, 2009), and the seeds of the F1 progeny should be tested as per component 3 of the sterility assessment. If seed production is significantly higher, then the risk assessment needs to be adjusted as such.

Discussion

For the regulation of cultivars and hybrids of invasive taxa, South Africa initially adopted an approach similar to the "Claimed to be innocent" [approach (5); Table 2], but subsequently moved to something between the "Negotiated guilt" [approach (4); Table 2], and "Guilty until proven innocent" [approach (3); Table 2]. Adopting a "Claimed to be innocent" approach is risky, and the approach may change over time as seen in the case of South Africa where taxa that were exempt, such as *Vinca major*, amongst others, were later banned (see Supplementary Appendix for other examples). Hence, we primarily recommend

https://doi.org/10.1017/inp.2024.24 Published online by Cambridge University Press

a “Guilty until proven innocent” approach for regulating cultivars or hybrids of invasive taxa at a national scale (i.e. all cultivars or hybrids where at least one related taxon is regulated should be regulated unless there is documented evidence that the cultivar/hybrid is not invasive). Sterile cultivars or hybrids of invasive plant species could be ideal candidates for such exemptions, but it is crucial that within the supporting evidence, sterility is not only appropriately assessed but also accurately defined.

Allowing for the independent regulation of cultivars and hybrids of invasive plant species, by exempting safe/non-invasive (usually synonymous with sterile) taxa that are underpinned by scientific evidence may be the most viable regulatory option. However, there will still remain other risks such as the misidentification of sterile cultivars or hybrids. Traders may knowingly or unknowingly label specific cultivars or hybrids with the names of the exempted taxa. Thus, it is recommended that procedures are put in place to aid with preventing this, such as routine genetic testing (e.g., DNA fingerprinting or sequencing approaches) and plant auditing. Further, public (and nursery customers) awareness would be needed for the approach to be widely acceptable and adopted. If the recommended regulatory approach is adopted nationally, there will be a push towards the patenting of sterile plants, making plant tracking easier. Finaly, generic pro-forma for reporting sterility based on observations and/or experiments (such as that presented in Figure 3 may assist with making the regulatory approach more easily implemented.

Recommendations

For the case of regulating cultivars or hybrids we primarily recommend evidence-based approaches such as the “Guilty until proven innocent” or the “Negotiated guilt” approaches. The “Guilty until proven innocent” approach is the most time-consuming and stringent, resulting in a relatively high number of taxa being banned, but is also the most evidence-based approach which seeks to minimize conflict of interest between stakeholders and legislators. This approach can be justified for regulating cultivars or hybrids because such taxa generally display similar traits to their parent genotypes, thus the precautionary principle still applies. Although this approach requires substantial information, it still allows for exemptions of cultivars or hybrids based on evidence gained from robust scientific experiments, making provision for “safe” taxa claims. This type of approach has been demonstrated in Oregon, USA, whereby 18 sterile cultivars of the invasive *Buddleja davidii* were deemed safe for trade/use (underpinned by scientific evidence), which served as a model for other states to follow (Contreras and McAninch 2013). Further, adopting a "Guilty until proven innocent" approach encourages research, which increases the output and volume of scientific knowledge pertaining to safe/non-invasive cultivars, as seen in the case of Florida (Wilson and Deng 2023). In the event that an "Innocent until proven guilty" approach cannot be successfully adopted, we recommend a "Negotiated guilt" approach. This approach does not require scientific evidence nor assessments, exemptions are based on known mechanisms and long-term observations which an independent body associated with the green industry is responsible for. However, it must be noted that the "Negotiated guilt" approach may lead to lengthy negotiations between regulators and users or inaccurate risk assessments of the exempted taxa.

Conclusion

Regulating infra- and inter- specific entities of taxa that are known to be invasive can be a complex task but is important if conflicts of interest between various stakeholders (primarily from industry) and regulators are to be resolved. South African regulation of infra- and inter-specific entities of invasive taxa is laudable, but the process can be more transparent and evidence-based. We recommend that for any country with cultivars or hybrids of non-native plant species, exemptions for infra- and inter-specific entities should be on the basis of risk analyses for those entities (e.g., Kumschick et al. 2020b for South Africa). Ideally risk analyses of the related entities should also be produced with clear explanation as to why the risk differs. Such exemptions should, we believe, be specified in official documentation so they are transparent.

https://doi.org/10.1017/inp.2024.24 Published online by Cambridge University Press

Acknowledgements

The South African Department of Forestry, Fisheries and the Environment (DFFE) are thanked for funding, noting that this publication does not necessarily represent the views or opinions of DFFE or its employees, and DFFE officials are thanked for their input regarding the regulations. DC acknowledges the Cape Peninsula University of Technology for research support. SK acknowledges the support of the Centre for Invasion Biology (CIB) at Stellenbosch University. Kay Montgomery is thanked for the valuable information provided pertaining to the South African horticultural industry. Katelyn Faulkner, Ashlyn Padayachee and Michael Cheek are thanked for comments on the manuscript.

Competing Interests

Competing interests: The author(s) declare none.

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https://doi.org/10.1017/inp.2024.24 Published online by Cambridge University Press

Table 1: The definition of sterility in plants may vary depending on the context and goal for which it is being used, thus it is crucial that sterility is accurately assessed and defined when the term is being used.

Term	Definition
Sterile	The term sterile when used within the context of plants in invasion science relates to plants with poor quantity or quality of pollen and seeds (Czarnecki et al., 2017; Deng et al., 2020) which limit or prevent sexual reproduction. This definition does not preclude asexual reproduction, and so plants that are sterile under this definition can still form invasive populations.
Presumed sterile	Anecdotal evidence from the South African horticulture industry suggests that the term "sterile" was used to define a plant that is unable to escape from cultivation (captivated/garden environment). Where this definition of "sterile" is used in the paper, we use the term "presumed sterile".

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Table 2: Six approaches for regulating cultivars and hybrids of invasive species from precautionary to reactive.

#	Approach	Explanation of approach	Evidence required	Number of entities banned	Effort to demonstrate safety	Ease of implementation	Expected number of listing errors
1	"Globally guilty by association"	All cultivars or hybrids related to any species that are invasive anywhere in the world are regulated It is based on the principle that the consequence of invasion is far higher than commercial interests. It is also consistent with the view that it is not possible to prove a particular genetic entity has, in perpetuity, lost the ability to become invasive	Demonstrated invasiveness of the cultivar or hybrid relative anywhere in the world	High	Low	Fairly simple, providing taxa related to the entity can be identified	High number of taxa banned which are low risk (false positives)

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#	Approach	Explanation of approach	Evidence required	Number of entities banned	Effort to demonstrate safety	Ease of implementation	Expected number of listing errors
2	"Nationally guilty by association"	If any entity is known to be invasive in the country adopting the regulation, all related cultivars and hybrids are banned It is based on the principle that the consequence of invasion is far higher than commercial interests. It is also consistent with the view that it is not possible to prove a particular genetic entity has, in perpetuity, lost the ability to become invasive	Demonstrated invasiveness of the cultivar or hybrid's relative in the same country adopting the regulation	High	Low	Fairly simple, providing taxa related to the entity can be identified	High number of taxa banned, which are low risk (false positives)

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#	Approach	Explanation of approach	Evidence required	Number of entities banned	Effort to demonstrate safety	Ease of implementation	Expected number of listing errors
3	"Guilty until proven innocent"	All entities are banned but cultivars or hybrids can be exempted if evidence from experiments demonstrate an acceptable level of risk of a harmful invasion Responsibility with industry to demonstrate non-invasiveness	Robust scientific experiments with large sample size and proper statistical analysis. Similar to pre-release safety assessment of biocontrol agents	High but also dependent on number of tests done and the results of those tests	High	Difficult. It is time and resource intensive	Initially high number of taxa banned that do not need to be (false positives), however, this approach allows for this number to be reduced over time

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#	Approach	Explanation of approach	Evidence required	Number of entities banned	Effort to demonstrate safety	Ease of implementation	Expected number of listing errors
4	"Negotiated guilt"	The level of guilt is set by agreements between regulators and users in the absence of strong information about invasiveness. Responsibility with regulator to demonstrate invasiveness OR industry to report invasiveness An independent body, e.g., associated with the green industry, might be made responsible for approving entities based on known mechanisms and observations	It is primarily based on observations, potentially from multiple sources. Ideally the putative mechanism for sterility or non-invasiveness of the cultivar/hybrid should be stated	Medium	Medium	Medium. Negotiations can be lengthy and time consuming; however it is inherently a consultative and inclusive approach	Possibly low number of taxa banned that do not need to be (false positives) and taxa allowed which are high risk (false negatives)

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#	Approach	Explanation of approach	Evidence required	Number of entities banned	Effort to demonstrate safety	Ease of implementation	Expected number of listing errors
5	“Claimed to be innocent”	A cultivar or hybrid is exempted when any stakeholder claims it to be non-invasive. It is based purely on anecdotal observations and good faith	No evidence required	Low	Low	Few resources needed	Possibly high number of taxa allowed which are high risk (false negatives)

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#	Approach	Explanation of approach	Evidence required	Number of entities banned	Effort to demonstrate safety	Ease of implementation	Expected number of listing errors
6	"Innocent until proven guilty"	All cultivars or hybrids are exempt from regulations unless there is evidence for invasiveness This approach argues that cultivars or hybrids differ significantly from the invasive species and therefore the invasiveness of the cultivar or hybrid must first be proved/demonstrated before it can be banned	Responsibility with regulator to provide scientific evidence demonstrating invasiveness	Low	Low	Does not require any resource for cultivars or hybrids to be regulated. However, evidence is required to prove invasiveness and the costs of producing such evidence might lie with society	Highest number of possible taxa allowed which are high risk (false negatives)

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Table 3: Taxa listed under the National Environmental Management: Biodiversity Act, Alien and Invasive Species Regulations for which there is or was provision to exempt sterile cultivars or hybrids. Column headings are as per DarwinCore terms where available. The scientificName was taken from Wilson (2024), with nomenclature checked therein against the Botanical Database of Southern Africa (BODATSA) and Plants of the World Online (POWO) during 2023. The vernacularName is as presented exactly in the NEM:BA A&IS Lists (including capitalisation). Other names used are either synonyms used in at least one version of the regulatory lists, synonyms specified in the regulatory listing [e.g., the NEM:BA A&IS List includes the following listing: "*Duranta erecta* L. (= *D. repens* L., *D. plumieri* Jacq.)"], or names misapplied in South Africa specified in the regulations (e.g., *Pyracantha fortuneana* was misapplied to *Pyracantha crenulata*). For full details see Supplementary Tables S1 - 2.

scientificName	**Other names used**	**vernacularName**	**Number of "presumed sterile" entities exempted**	
			2014	**2020**
Acer negundo L.	none	Ash-leaved maple, Box elder	0[*a]	0[*a]
Ageratum houstonianum Mill.	none	Mexican ageratum	6	6
Berberis thunbergii DC.	none	Japanese barberry	10	10
Buddleja davidii Franch.	none	Chinese sagewood, Summer lilac	7	7
Canna indica L.	none	Indian shot	0	no provisions
Catharanthus roseus (L.) G.Don	none	Madagascar periwinkle	77	77
Cenchrus setaceus (Forssk.) Morrone	*Pennisetum setaceum* (Forssk.) Chiov.	Fountain grass	5	5

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Cestrum L.	none	Cestrum species	0	no provisions
Coreopsis lanceolata L.	none	Tickseed	0	no provisions
Cortaderia selloana (Schult.) Asch. & Graebn.	none	Pampas grass	2	no provisions
Duranta erecta L.	*Duranta repens* L. | *Duranta plumieri* Jacq.	Forget-me-not-tree, Pigeon berry	6	6
Gleditsia triacanthos L.	none	Honey locust	6	no provisions
Hedera canariensis Willd.	*Hedera helix* L. subsp. *canariensis* (Willd.) Cout.	Canary ivy, Madeira ivy, Algerian ivy	3	3
Hedera helix L.	*Hedera helix* L. subsp. *helix*	English ivy	20	20
Ipomoea indica (Burm.) Merr.	*Ipomoea congesta* R.Br.	Blue morning glory	0	no provisions
Ipomoea purpurea (L.) Roth	none	Purple morning glory	0	no provisions
Ligustrum lucidum W.T.Aiton	none	Chinese wax-leaved privet	1	no provisions
Ligustrum ovalifolium Hassk.	none	Californian privet	1	1
Limonium sinuatum (L.) Mill.	none	Statice, Sea lavender	11	no provisions

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Metrosideros excelsa Sol. ex Gaertn.	*Metrosideros tomentosa* A.Rich.	New Zealand Christmas tree	6	no provisions
Melaleuca viminalis (Sol. ex Gaertn.) Byrnes	*Callistemon viminalis* (Sol. ex Gaertn.) G.Don	Weeping bottlebrush	6	6
Morus alba L.	none	White mulberry, Common Mulberry	0	no provisions
Murraya paniculata (L.) Jack	*Murraya exotica* L.	Orange Jessamine	1	1
Nephrolepis cordifolia (L.) C.Presl	*Polypodium cordifolium L.*	Erect sword fern|Ladder sword fern	2	no provisions
Nephrolepis exaltata (L.) Schott	*Polypodium exaltatum* L.	Sword fern, Boston sword fern	11	11
Nerium oleander L.	none	Oleander	1 + All double flowering cultivars	no provisions
Opuntia ficus-indica (L.) Mill.	*Opuntia megacantha* Salm-Dyck	Mission prickly pear, Sweet prickly pear	4	4
Opuntia robusta H.L.Wendl. ex Pfeiff.	none	Blue-leaf cactus	0	0
Pyracantha angustifolia (Franch.) C.K.Schneid.	none	Yellow firethorn	0	no provisions
Pyracantha coccinea M.Roem.	none	Red firethorn	0	no provisions

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Pyracantha crenulata (D.Don) M.Roem.[*b]	*Pyracantha crenatoserrata (*Hance) Rehder | *Pyracantha fortuneana* | *Pyracantha crenulata (D.Don)* M.Roem *var.* rogersiana | Pyracantha *rogersiana* (A.B.Jacks.) Chitt.	Chinese firethorn, Broad leaf firethorn, Himalayan firethorn	0	no provisions
Pyracantha koidzumii (Hayata) Rehder	none	Formosa firethorn	0	no provisions
Vinca major L.	none	Greater periwinkle	2	no provisions
Vinca minor L.	none	Lesser periwinkle	4	4

[*a] = It is unclear if there were any submissions of sterile cultivars/hybrids for *Acer negundo* as this taxon does not appear in the unofficial consultative list (Supplementary Table S1), however there have been provisions for exemptions for this taxon since 2014.

[*b =] *Pyracantha crenatoserrata* is a recognised synonym of *Pyracantha crenulata* (Plants of the world online) but is listed separately in the NE: MBA regulations.

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Table 4: Anecdotes from the green industry regarding "presumed sterile" cultivars or hybrids. It is interesting to note existing impressions that exist among members of the Green industries regarding certain cultivars and hybrids perceived to be "non-invasive" and safe for trade. These perceptions are anecdotal evidence based on long term observations. Some of these are briefly discussed in this table.

Plant/term	Discussion
Variegated forms	A prevalent notion is that variegated forms of known invasive species are sterile. For example, the variegated form of *Vinca major* was considered sterile and suitable for trade (2014-2017) in South Africa, however this was amended as the cultivar was observed to spread vegetatively. Variegated forms might fix less carbon than non-variegated forms and so have slower growth rates, but we know of no evidence that confirms sterility in variegated forms.
Bonsai plants	Due to small stature, the fecundity of the bonsai plants is limited. Bonsai plants are usually maintained indoors or in a highly managed environment, unlike many garden plants that can occur on the edges of gardens, or are not well maintained. As such, the possibility of a bonsai becoming invasive is very low. However, in principle, even a few viable seeds could lead to the start of a new population. Since dwarfness in bonsai is usually human-induced rather than genetic , the progeny from bonsai can become full-sized trees.
Purple Fountain grass (*Pennisetum* x *advena* "Rubrum")	Fountain grass is regulated in South Africa, but the red/purple cultivars have very high ornamental value and are considered sterile. They are still sold in the nurseries as a "presumed sterile" cultivar in the country. However, we know of no published evidence demonstrating the sterility of this cultivar. The issue of "presumed sterile" cultivars does not only pertain to cultivars of Purple Fountain grass in South Africa but extends to 15 other taxa (as shown in Table 1) encompassing a total of 157 "presumed sterile" cultivars/hybrids in South Africa.

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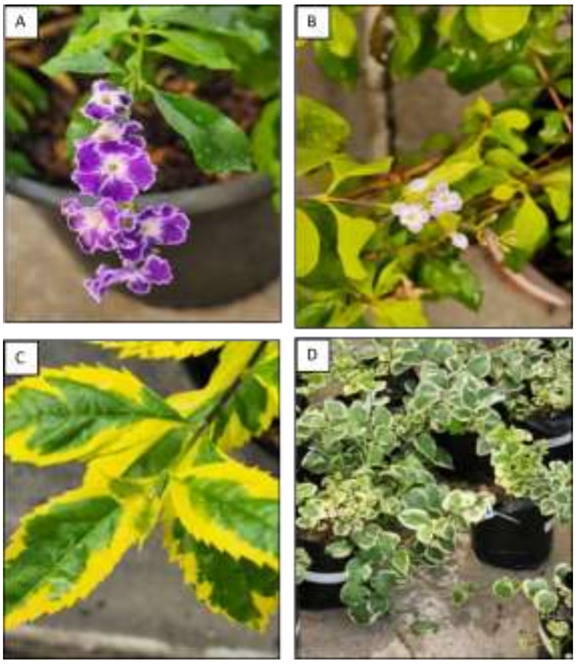

Figure 1: Photo panel illustrating examples of "presumed sterile" cultivars in South: A) *Duranta erecta* "Sapphire Showers"; B) *Duranta erecta* "Sheena's Gold"; C) *Duranta erecta* "Goldmine"; D) *Vinca major* "Variegata".

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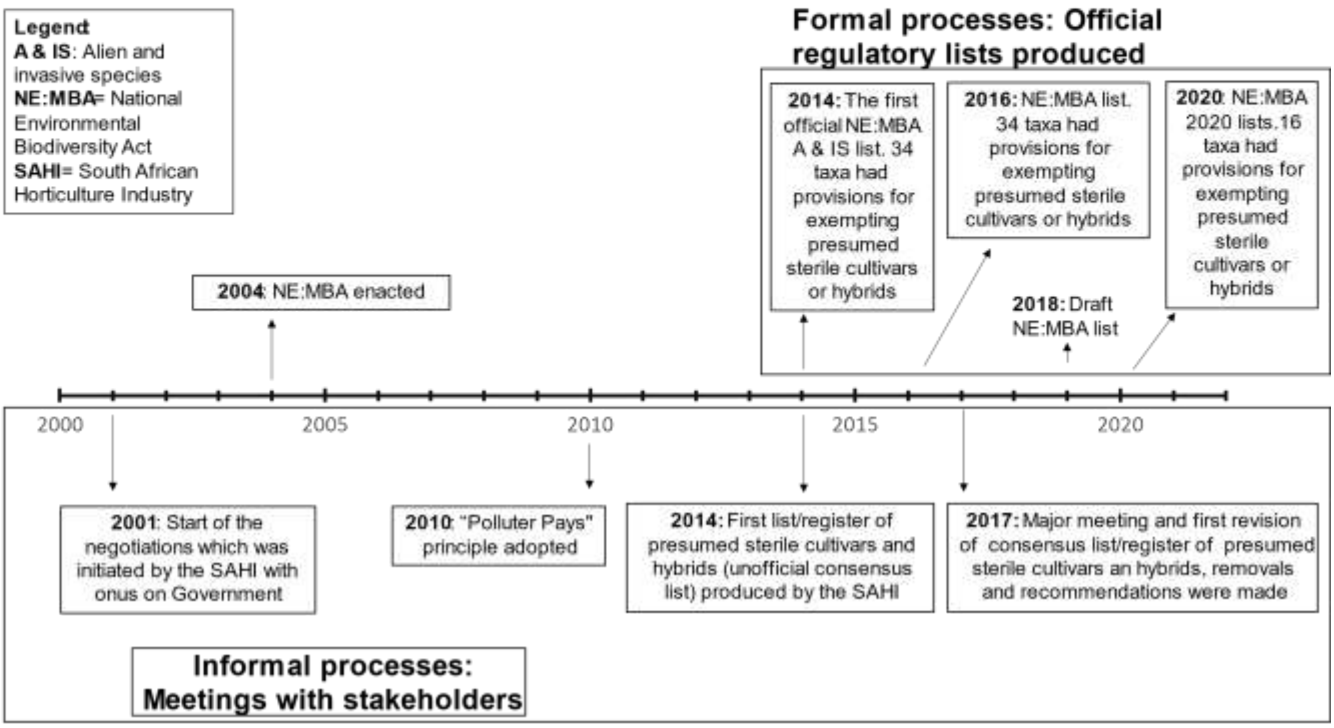

Figure 2: Timeline displaying the major events of the negotiations between the South African Horticultural Industry (SAHI) and the Department of Forestry, Fisheries and the Environment (DFFE) regarding "presumed sterile" cultivars of invasive plants in South Africa. For a detailed timeline of the NEM:BA A&IS Regulations and Lists see Wilson & Kumschick (2024).

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Pro- forma (template): **What should a sterility assessment for cultivars or hybrids of invasive species include?**

1a. Does the entity produce flowers? (Yes/No/Not studied/NA)
Notes on evidence: examples include long-term flower evaluation with greenhouse or common-garden experiments.

1b. Does the entity produce fruit? (Yes/No/Not studied/NA)
Notes on evidence: examples include long-term fruit evaluation with greenhouse or common-garden experiments

2. Does the entity produce viable pollen? (Yes/No/Not studied/NA - if no flowers)
If yes, how does production and/or viability compare with other entities?
Notes on evidence: examples include pollen viability and germination experiments.

3. Does the entity produce viable seeds? (Yes/No/Not studied/NA)
If yes, how does production and/or viability compare with other entities?
Notes on evidence: examples include seed viability and germination experiments.

4. **Does the entity reproduce vegetatively?** (Yes/No/Not studied/NA)
If yes, how does how does it compare to other entities?
Notes on evidence: examples include cuttings propagation experiments along with vigour and growth rate assessments.

5. Does the entity become fertile/remain sterile after out-crossing? (Yes/No/Not studied/NA):
Notes on evidence: examples include cross-pollination experiments to determine seed set and seed viability.

Overall conclusion
Example: The entity is dependent on seed production for it to become invasive. The entity produces fertile seeds but at a much lower level than parental stock. The likelihood and rate of an invasion of the entity is therefore likely to be substantially lower than parental stock but it is not clear that the overall risk is below an acceptable threshold.

Figure 3: A generic pro-forma for reporting sterility based on observations and/or experiments on: flowering, fruiting, pollen, and seeds; the potential for vegetation propagation; and the potential for genetic changes (including hybridisation and reversion to fertility).

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List of supplementary material:

Supplementary Table S1: Unofficial consultative consensus list of *"sterile"* cultivars and Hybrids (2014).

Supplementary Table S2: Unofficial consultative consensus list of *"sterile"* cultivars and Hybrids (2022).

Supplementary Appendix: Proposals by Government to the Green Industry (Information obtained from key stakeholder engagement meeting held on the 14th of February 2017)

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