

# Identifying safe cultivars of invasive plants: six questions for risk assessment, management, and communication

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## Abstract

The regulation of biological invasions is often focussed at the species level. However, the risks posed by infra- and inter-specific entities can be significantly different from the risks posed by the corresponding species, to the extent that they should be regulated and managed differently. In particular, many ornamental plants have been the subject of long-term breeding and selection programmes, with an increasing focus on trying to develop cultivars and hybrids that are less invasive. In this paper, we frame the problem of determining the risk of invasion posed by cultivars or hybrids as a set of six questions that map on to the key components of a risk analysis, viz., risk identification, risk assessment, risk management, and risk communication. 1) Has an infra- or inter-specific entity been proposed as “safe to use” despite at least one of the corresponding species being a harmful invasive? 2) What are the trait differences between the proposed safe alternative and its corresponding invasive species? 3) Do the differences in traits translate into a difference in invasion risk that is significant for regulation? 4) Are the differences spatially and temporally stable? 5) Can the entities be distinguished from each other in practice? 6) What are the appropriate ways to communicate the risks and what can be done to manage them? For each question, we use examples to illustrate how they might be addressed focussing on plant cultivars that are purported to be safe due to sterility. We review the biological basis of sterility, methods used to generate sterile cultivars, and the methods available to confirm sterility. It is apparent that separating invasive genetic entities from less invasive,

but closely related, genetic entities in a manner appropriate for regulation currently remains unfeasible in many circumstances – it is a difficult, expensive and potentially fruitless endeavour. Nonetheless, we strongly believe that an *a priori* assumption of risk should be inherited from the constituent taxa and the onus (and cost) of proof should be held by those who wish to benefit from infra- (or inter-) specific genetic entities. The six questions outlined here provide a general, science-based approach to distinguish closely-related taxa based on the invasion risks they pose.

### Keywords

cultivars, hybrids, infra-specific genetic entities, invasive species, non-invasive cultivars, ornamental plants, seedless cultivars, sterility

## Introduction

Invasion is a population-level phenomenon (Petit 2004; Zenni et al. 2014). Nonetheless, most regulatory policies focus implicitly or explicitly at the species-level. Consequently, the enormous variation that exists at infra-specific levels is often not considered in regulatory frameworks. The inability to recognise differences below the species rank may lead to serious underestimation or overestimation of invasion risk (Gordon et al. 2016). For example, infra-specific entities can vary in the bioclimatic niches they occupy in their invasive ranges (Thompson et al. 2011; Gotelli and Stanton-Geddes 2015), their host-specificity (Goolsby et al. 2006), and the impacts they cause (Novoa et al. 2018). Likewise, invasions by inter-specific taxa are also very important: hybridisation is one of the major impacts caused by biological invasions (Huxel 1999; Yakandawala and Yakandawala 2011). Therefore, it is vital that policy and regulation can adequately address invasion risk at levels other than the species.

These issues are particularly significant in the context of horticulture. The introduction of plants as ornamentals constitutes a major pathway for invasive plants across the globe (Bell et al. 2003; van Kleunen et al. 2018). Many of the traits that are important for horticultural purposes can also promote invasiveness (Reichard and Hamilton 1997; van Kleunen et al. 2018), for example, the formation of dense thickets, profuse flowering, high fruit set, and wide environmental tolerance (Knight et al. 2011; van Kleunen et al. 2018). In contrast, some horticulturally-desirable traits lead to reduced competitive ability; for example, variegated leaves in plants can have lower photosynthetic performance than non-variegated leaves (Gaskin and Kazmer 2009). Horticulture, therefore, creates very particular ecologically-relevant biases in infra-specific and inter-specific genetic entities. Moreover, many ornamental plants have been subjected to artificial selection and breeding programmes to enhance specific attributes of interest (Reichard and White 2001; van Kleunen et al. 2018), leading, in some cases, to high diversity of genetic entities below and above the species rank. This can have direct consequences for the likelihood of an invasion. For example, above the species rank, hybridisation between two or more species or even genera can promote genetic diversity and increase invasiveness (Culley and Hardiman 2009; Gaskin and Kazmer 2009; Klonner et al. 2017). Below the species rank, cultivars of a species can differ in traits

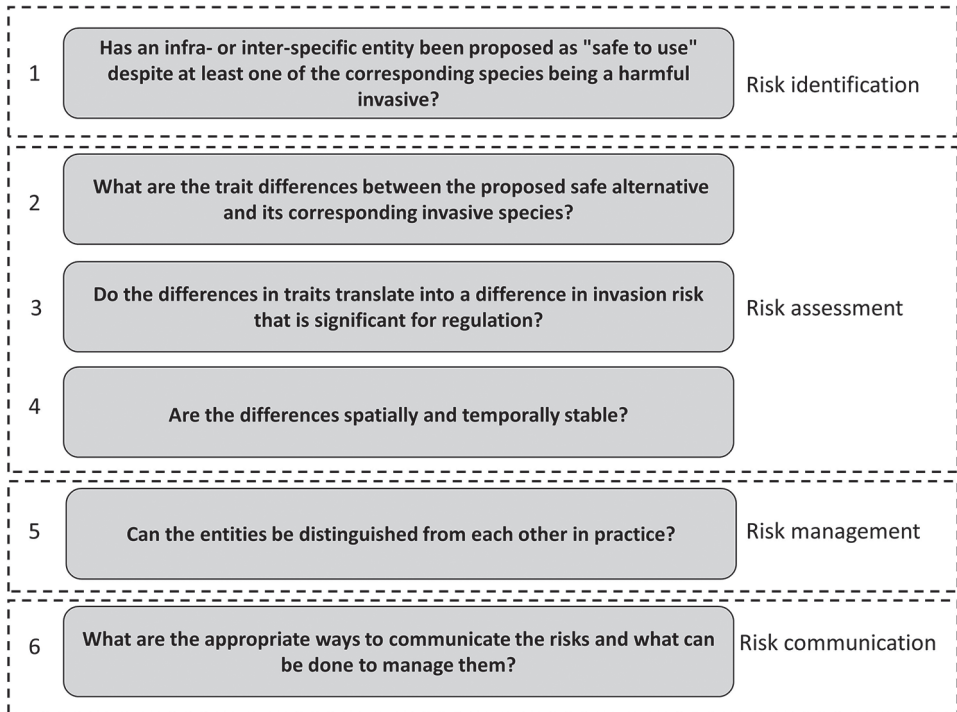
such as allelopathy (Alsaadawi et al. 2012; Al-Bedairy et al. 2013) and herbicide tolerance (Sterling et al. 2004). Horticulture, therefore, creates very particular ecologically-relevant biases in infra-specific and inter-specific genetic entities.

In response to the risks of biological invasions, several countries have enacted legislation to regulate the use and trade of invasive plant species. Many of these regulated species are, however, of great ornamental value, and so such regulations cause economic losses and directly impinge on individual rights (Wirth et al. 2004). Consequently, there has been pressure to either exempt particular genetic entities that are naturally “safe” or “non-invasive” or to develop cultivars that are more environmentally sustainable (Guo et al. 2004; Freyre et al. 2014).

A specific case in point is South Africa’s National Environmental Management: Biodiversity Act, Alien and Invasive Species Regulations of 2014 (Department of Environmental Affairs 2014), hereafter referred to as the NEM:BA A&IS Regulations. In an attempt to balance the goal of environmental protection with those of the horticultural industry, the regulations have provision to exempt infra- or inter-specific entities. Out of 379 plant taxa listed in the 2016 revised list, sterile cultivars or hybrids are not listed for 32 taxa, spineless varieties of two cactus species are exempted, and sterile forms of *Pinus elliotti* are regulated differently from fertile forms (Department of Environmental Affairs 2016; Suppl. material 1: Table S1). While excluding sterile cultivars or hybrids is a laudable effort to reduce potential conflicts, the regulations do not provide any guidance on how this is to be implemented, and only in one case, *Duranta erecta* “Sheena’s Gold”, is an acceptable cultivar specifically named. The regulations also implicitly assume that sterility is a necessary and sufficient condition to reduce invasiveness and impact. However, some of the most damaging invasive plants are predominantly sterile in their invasive range [e.g. water hyacinth (*Eichhornia crassipes*); (Zhang et al. 2010)], conversely, infra- or inter-specific entities might still be fertile, but either the reduction in fertility or changes in other traits mean that they pose an acceptable level of risk. Finally, the risk-reducing trait might not be stable, and so a ‘safe’ cultivar could revert to an ‘invasive’ plant [e.g., there is some indication that the spineless non-invasive cacti exempted under the NEM:BA A&IS Regulations might readily revert to spiny invasive forms (Novoa et al. 2019)]. While the consideration of sub-specific entities has been included in a recent risk analysis framework that is being used to provide scientific recommendations for the NEM:BA A&IS Regulations (Kumschick et al. 2020), the framework does not yet include a detailed protocol for how to analyse the relative risk of infra- or inter-specific entities.

### **Six questions to serve as a guide to differentiate “safe” cultivars from “risky” relatives.**

To clarify the issue of how to separate “safe” cultivars from “risky” relatives, we developed a set of six questions (Fig. 1). The questions are framed so they align with the general steps of a risk analysis, i.e., risk identification, risk assessment, risk management, and risk communication.



**Figure 1.** Six questions that should be answered if “safe” cultivars are to be differentiated from “risky” relatives in regulations on biological invasions. The questions align with the constituent parts of risk analysis as indicated by the dotted boxes. Each of the questions is explained in further details in the main text.

*Question 1: Has an infra- or inter-specific entity been proposed as “safe to use” despite at least one of the corresponding species being a harmful invasive?* To minimise the risk of invasion from known invasive ornamental species, the use of non-invasive and sterile forms has been promoted. Question 1 concerns identifying and specifying this problem. Is there a cultivar of an invasive ornamental species that is deemed to be safe? Is there sufficient demand for this cultivar to warrant answering the other questions? It is essential to assess the invasion risk of a supposedly non-invasive genetic entity in the context of the invasiveness of the closely-related invasive taxa or parent invasive taxa (Table 1). The list of (potentially safe) cultivars can be obtained from published cultivar names (e.g. International Cultivar Registration Authorities), nursery catalogues, and consultation with stakeholders such as plant breeders and wholesale nurseries.

*Question 2: What are the trait differences between the proposed safe alternative and its corresponding invasive species?* This question refers to measurable differences that could help us to characterise and differentiate between cultivars and the known invasive entity. These differences could either be due to underlying genetic differences or could be induced due to environmental factors. The traits could include vegetative traits (e.g., leaf size, presence of variegated leaves, presence of thorns and spines, height, and growth form) or reproductive traits (e.g., flower colour, phenology or number of fruits

**Table 1.** Selected case studies in which sterile cultivars and hybrids were specifically generated as an alternative to known invasive stocks. Details of the cultivar name, method used to generate the cultivar or hybrid, biological cause of sterility, and the commercial purpose of generating the sterile cultivar or hybrid are detailed below.

Taxa	Cultivar name(s)	Method	Cause of sterility	Purpose	Reference
Citrus	NA	Cybridisation	Cytoplasmic male sterility	Development of seedless fruits	Guo et al. (2004)
<i>Euonymus alatus</i>	Compactus	Ploidy alternation: Triploid plant generation	Uneven division of chromosomes	Development of sterile ornamental	Thammina et al. (2011)
<i>Lantana camara</i>	UF-T3 UF-T4	Interploid Hybridisation	Highly reduced pollen fertility and seed set (with seed germination highly reduced for UF-T3 and zero for UF-T4)	Development of sterile ornamental	Czarnecki et al. (2012)
<i>Ruellia simplex</i>	R10-102 (Mayan Purple) R10-108 (Mayan White) R12-2-1 (Mayan compact purple)	Interploid hybridisation and induced polyploidy using oryzalin	Fruitless and low pollen viability (R10-102 and R10-108), and both female and male sterility (R12-2-1)	Development of sterile ornamental	Rosanna Freyre et al. (2012)
<i>Verbena x hybrida</i>	SS SC	Mutation by heavy-ion beam	Non functional male and female gametes in SS and self-incompatibility in SC	To halt senescence and increase flowering duration of the plants	Kanaya et al. (2008)

or seeds). In some cases, underlying genetic differences leading to sterility may not be easily detected from phenotypic traits and, therefore, further examination of cytological and genetic differences could be necessary.

*Question 3: Do the differences in traits translate into a difference in invasion risk that is significant for regulation?* In question 3, we relate the observed differences (seen in question 2) to differences in the level of invasion risk posed and whether any such differences in risk mean that the taxa sit on different sides of a regulatory decision point, i.e., specimens with one set of physical properties pose an acceptable level of risk, while others do not. The observable differences in traits of the related genetic entities may lower the invasion risk only if the fecundity is directly or indirectly lower than the known invasive form. Traits that are directly related to fecundity include pollination, length of flowering time, number of flowers, fertilisation, seed production, germination success, survival rate, and vegetative reproduction. Traits that indirectly affect fecundity include allelopathic potential, mycorrhizal mutualisms, and herbivore deterrence due to the presence of thorns or chemicals. To detect differences in fecundity between different genetic entities, it is necessary to grow them in the same common garden environment and monitor long term. Ideally, the fecundity (or offspring survival) should be so low that population growth rate is negative (Knight et al. 2011).

*Question 4: Are the differences spatially and temporally stable?* Question 4 concerns whether the changes in the observable traits are stable and no reversal to the parental conditions is likely (see examples in Table 2). The changes should ideally be genetically fixed and not induced by environmental factors or due to short term epigenetic modifications. Even genetic changes could be reversed due to occasional outcrossing with other genotypes. Therefore, long term common garden experiments under differ-

**Table 2.** Selected examples of cultivar evaluation. Details of the specific method used for evaluation, number of years the evaluation took, and the main result are tabulated below. This Table corresponds to the risk assessment section (questions 2–4) of the conceptual framework proposed (Fig. 1).

Taxa	Method(s) of evaluation	Duration (Years)	Main Results	Reference
<i>Berberis thunbergii</i>	Common garden experiments Seed germination experiments	4–5	Out of 46 cultivars, most cultivars produced seeds. Cultivars that failed to produce seeds initially produced seed after the plants matured for 4–5 years. None of the cultivars can be considered non-invasive.	Brand et al. (2012)
<i>Euonymus alatus</i>	Common garden experiment Open seed germination Establishment experiment	3	None of the cultivars was completely seedless and failed to germinate. Habitat had a strong influence on seed germination and establishment.	Brand et al. (2012)
<i>Lantana camara</i>	Cytology Pollen staining Common garden experiments	3	All the cultivars produced viable pollen. Almost all cultivars produced viable seeds. Even sterile triploid cultivars produced seeds when allowed to cross pollinate with diploid cultivars. None of the plants were truly sterile.	Spies and du Plessis (1987)
<i>Nandina domestica</i>	Common garden experiments Seed germination	1–2	Large cultivars produced more viable seeds than dwarf cultivars. Seed viability was close to zero for some cultivars which were hence recommended for use.	Knox and Wilson (2006)
<i>Ruellia tweediana</i>	Common garden experiment Seed germination	1–2	All the cultivars were capable of producing viable seeds that germinated. Environmental conditions (light and temperature) influenced the fecundity.	Wilson and Mecca (2003)
<i>Spiraea japonica</i>	Common garden experiments Pollen and seed germination Pollination experiments Flow cytometry	1	Three sterile cultivars were identified that did not produce any viable seeds and had very poor pollen germination. Sterility was not related to polyploidy.	Wilson and Hoch (2009)
<i>Viburnum opulus</i>	Field assessment and germination experiments.	2	All cultivars produced seeds, but varied in amount. Poor germination in open field sites compared to green house	Conklin and Sellmer (2009)

ent experimental conditions should be performed. In order to ensure that fertility does not revert, cross-pollination experiments should be performed between the different non-invasive genetic entities under consideration and the known invasive form. However, ultimately a regulator is interested in whether reversion is likely in the context of where and when (and in what numbers) the entity will be used. If a cultivar is very popular and widely planted, an extremely rare reversion is more likely to happen than for unpopular cultivars, and on-going monitoring might be advisable (see Question 6).

*Question 5: Can the entities be distinguished from each other in practice?* Question 5 refers to the need that, if the regulation is to be implemented, the safe cultivar must be readily distinguishable from its invasive relatives. This is particularly important for management and regulation so that non-invasive genetic entities can be exempted and monitored. Phenotypic differences might depend on growing conditions, and so other assays (Table 3) should be performed whenever necessary. Molecular markers for specific cultivars could be developed so that they can be readily detected.

*Question 6: What are the appropriate ways to communicate the risks and what can be done to manage them?* Finally, question 6 requires a mechanism by which recommendations are developed together with stakeholders in a transparent and inclusive manner (e.g., Novoa et al. 2018). This should be based on the results from the previous questions.

**Table 3.** Selected examples of cultivar identification using different techniques. In order to ensure effective regulation, the cultivar has to be distinguishable from the invasive ones. This Table corresponds to the risk management section of the conceptual framework (question 5) (Fig. 1).

Taxa	Method used	Details of the study	Reference
<i>Castanea sativa</i>	Pollen morphology and germination	Characterisation of sterile and fertile pollen based on pollen morphology.	Mert and Soyly (2007)
Kangaroo Paws: <i>Anigozanthos</i> and <i>Macropidia</i>	Plastid DNA sequencing	Construction of phylogenetic tree based on plastid DNA confirmed hybrid origin of invasive population and other commercially available cultivars.	Le Roux et al. (2010)
<i>Prunus persica</i>	Molecular markers (RAPDs)	Marker based identification of genes responsible for pollen sterility (Ps/ps).	Jun et al. (2004)
Purple-leaved Japanese barberry: <i>Berberis thunbergii</i> var. <i>atropurpurea</i> and Green leaved <i>Berberis thunbergii</i>	Shade treatments in common garden	The purple leaves of <i>Berberis thunbergii</i> var. <i>atropurpurea</i> become green when grown under shade. Therefore, they cannot be easily distinguished from green-leaved <i>Berberis thunbergii</i> under shaded conditions.	Lehrer and Brand (2010)

### The basis of sterility and how to demonstrate it

Ultimately, the risk posed by a biological invasion is a function of population growth rate, spread rate, and subsequent impacts. Sterility in and of itself is neither a necessary nor sufficient condition to prevent damaging invasions. However, for some taxa (those that do not show asexual reproduction in particular) it is a sufficient condition and one that is particularly relevant to the development of “safe” cultivars from “risky” relatives. In this section, we review the biological bases of sterility and the different methods that have been developed to produce sterile cultivars. Furthermore, we discuss the different methods used to evaluate how “safe” a cultivar is. In each case, we highlight and discuss the links between these issues and how they address the six questions posed in Figure 1.

### The biological bases of reduced fecundity and sterility

Fecundity refers to the total number of viable offspring an individual produces over a lifetime. In most plants, fecundity is measured by viable seed production. It is crucial to understand the developmental processes associated with reduced fecundity when studying invasive plants and their apparently less invasive cultivars—in the presented framework, this relates to questions 2–4. In this section, we discuss several mechanisms that can cause low fecundity in plants (viz., cytoplasmic male sterility, pollen – stigma incompatibility, developmental changes, cytological incompatibility, and abortion of embryos) and note the consequence of these for identifying “safe” cultivars.

*Cytoplasmic male sterility:* The inability of plants to produce functional pollen due to cytoplasmic male sterility is a well-known phenomenon across different groups of angiosperms and is attributed to cytoplasmic factors that are maternally inherited through mitochondria (Schnable and Wise 1988). Specific peptides produced in mitochondria of male-sterile plants are capable of interfering with normal pollen development. These peptides are known to reduce ATP production, enhance

the production of reactive oxygen species and cause cytotoxicity (Horn et al. 2014). Interestingly, fertility can be automatically restored (e.g. *Petunia*) in such sterile plants by the action of specific nuclear genes that express proteins which regulate the degradation of mitochondrial proteins responsible for male sterility or by affecting mitochondrial DNA organisation (Gillman et al. 2007, Horn et al. 2014). Therefore, the sterility of pollen may not be a permanent phenomenon and fertility could potentially be restored in male-sterile plants.

*Pollen-stigma incompatibility:* Fertilisation can occur only when a compatible type of pollen lands on the stigma. Specific proteins are known to mediate the recognition of compatible pollen with the stigmatic papillae (Mattsson et al. 1974; De Nettancourt 1997). For example, in *Brassica* self-incompatibility has been detected due to the presence of specific glycoproteins (Luu et al. 1999). In dioecy (separate male and female plants), reproductive assurance cannot be obtained through the breakdown of self-incompatibility. Interestingly, dioecious species can be as invasive as monoecious species (Daehler 1998). This could be due to leaky dioecy i.e. the ability of a dioecious species to self-fertilise by the presence of flowers of both sexes on a single plant (Venkatasamy et al. 2007). Another mechanism for incompatibility is a physiological incompatibility system that is associated with tristylly. Tristylly is a rare breeding system that ensures optimal seed production and gene flow through cross-pollination since each plant possesses only one of three tristylous morphs (Ornduff 1966). In the tristylous *Pontederia cordata* L. (Pontederiaceae), although self-incompatibility is strongest in the short-styled flowers, it can occasionally break down leading to seed formation. Interestingly, preliminary field observations throughout its invasive range in South Africa have only recorded short-styled morphed flowers and no seed production. A cultivar might appear to be infertile, but will set seed if pollinated by compatible pollen. Multiple introductions of different genotypes increase the chances of restoring fertility in such cases. This suggests that unconditional sterility can only be confirmed conducting outcrossing experiments using a wide diversity of genotypes.

*Modifications of floral parts:* Differentiation of floral parts is delicately orchestrated by differential gene expression. Mutations in the genes leading to interference with gene expression can lead to the formation of incomplete or defective flowers. However, interestingly, these modifications are sometimes desired traits in the horticultural industry. For example, in some cultivars of petunia, stamens are converted into an additional row of petals or sepals (van der Krol and Chua Nam Hai 1993). Although the intention behind the development of such cultivars might be purely aesthetic, they might lead to reductions in fecundity, thus potentially lowering invasion risk.

*Cytogenetic anomalies:* Plants can also fail to produce outcrossed seeds for cytological reasons. For example, plants with an odd level of ploidy often fail to produce viable gametes due to abnormal laggard formation during meiosis. However, apomixis can restore fecundity in such cases (Noyes 2007). *Ageratina adenophora* is an example of a highly-invasive triploid Asteraceae that can vigorously reproduce by



virtue of its apomictic seeds (Baker 1974; Noyes 2007). Additionally, some instances of successful sexual reproduction in triploid cultivars have been recorded in *Lantana camara* (Spies & du Plessis, 1987). Therefore, the use of triploid cultivars should be advocated with caution.

*Abortion of fruits and seeds:* is a well-known phenomenon that has been observed in a diverse group of vascular plants (Ganeshiah and Uma Shaanker 1994; Arathi et al. 1996). Besides cytogenetic anomalies, several other genetic factors might cause abortion of embryos in seed plants. Maternal genotypes in *Pinus sylvestris* determine the seed abortion rate (Kärkkäinen et al. 1999). In *Dedeckera*, accumulation of a lethal genetic load in the populations can lead to developmental abnormalities which, in turn, lead to low viability and low germinability of seeds (Wiens et al. 1989). In the context of invasive ornamental plants, it would be desirable to grow cultivars that have inherent genetic factors that inhibit seed development rather than cultivars in which sterility has been caused by environmental cues.

*Exogenous factors:* Sub-optimal environmental conditions can reduce the number of seed and fruit set in plants (Lee 1988). Specific chemical triggers are also known to promote selective abortion of seeds in certain plants (Ganeshiah and Uma Shaanker 1994). Additionally, the absence of favourable biotic interactions such as specialised pollinators in figs and orchids can lead to a seedless condition (Richardson et al. 2000). However, such exogenous factors will only limit invasiveness as long as they are in place and so might require close control if an invasion is to be prevented.

## Methods to generate sterile cultivars

Many mechanisms promoting sterility or reduced fecundity discussed above can be induced or enhanced via plant breeding or molecular techniques. A wide array of such techniques to produce cultivars is currently available (see Table 1 for some case-studies). A thorough understanding of these techniques and how they induce sterility or reduce fecundity is important to understand and use questions 2–4 to distinguish between “safe” cultivars and “risky” relatives (Fig. 1).

*Traditional breeding:* Traditional plant breeding methods are relatively inexpensive, but they require great effort and time to screen for individuals with desired traits. Therefore, recent advances in biotechnology have been explored to produce sterile forms of invasive plants (Vining et al. 2012). Directional natural selection usually prefers the more fecund genotypes over the less fecund genotypes; as a result, the less fecund are often eliminated from the gene pool. For example, sterile triploids in nature are often lost due to natural selection. Traditional breeding methods (i.e., careful observation, artificial selection, and propagation by vegetative means) can, however, still be used to produce sterile or less fecund cultivars.

*Induced polyploidy:* Induction of polyploidy by the use of antimitotic agents (such as colchicine and oryzalin) has been widely used by plant breeders, as they are rela-

tively inexpensive and technically feasible. Induced polyploidy has often been used in conjunction with hybridisation techniques to produce sterile individuals (Vining et al. 2012, Freyre 2016).

*Hybridisation:* Hybridisation in plants may be possible between cultivars, species and even genera. Hybridisation between genetic entities with different ploidy levels often leads to sterility due to chromosomal abnormalities leading to interference with normal meiotic cell division. For example, hybridisation between hexaploid and diploid forms can result in the formation of triploid progenies which are generally sterile due to an odd ploidy level. However, in rare cases, reversal of sterility may result from cross-pollination with fertile forms (Spies and du Plessis 1987). Plants with odd chromosome numbers can also be raised from endosperm culture (Vining et al. 2012). Hybridisation experiments can, however, also potentially increase the vigour of the resulting hybrid (Ellstrand and Schierenbeck 2000), thus posing a greater risk if the sterility is accidentally reverted or if fertility is not a requirement for invasiveness. Cybridisation or somatic hybridisation is the process of producing hybrids between two sexually-incompatible individuals by fusing the protoplasm of two cells. This technique allows efficient transfer of cytoplasmic male sterility determined by mitochondrial genes (Guo et al. 2004).

*Induced mutation:* Mutation breeding using radiation (e.g., from x-rays, ion-beams or gamma-rays) or chemical mutagens [e.g. ethylmethanosulphonate (EMS)] is a popular technique in the toolbox of plant breeders for producing desired traits, including sterile and non-invasive forms (Broertjes and Dejong 1984; Kanaya et al. 2008).

*Recombinant DNA technology:* Transgenic techniques/recombinant DNA techniques can also potentially be used to transfer the genes of interest, leading to sterility (Vining et al. 2012). Such target genes could be genes responsible for cytoplasmic male sterility such as *cox2* gene (cytochrome c oxidase subunit 2) and T-urf13 gene (Štorchová 2017). However, such techniques should be used with caution, especially while working on invasive species, particularly if there is a risk of hybridisation with related native varieties or species.

## Evaluation of sterile cultivars

Different methods have been used to assess the sterility of cultivars or hybrids (some key examples are listed in Table 2). These techniques range from relatively simple and easy to conduct assays (e.g. pollen staining, germination and compatibility tests, and seed viability and germination tests) to more advanced techniques relying on (e.g., molecular markers, cytological examination of chromosomes, long term common garden experiments, and pollination experiments). Here, we discuss some of these.

*Pollen viability tests:* Pollen staining and germination tests evaluate the quality of pollen produced by the plant. Pollen is stained with cotton blue solution and the number of viable pollen (i.e., that is stained) is counted under a microscope (Czarnecki et al. 2012). Enzymatic induction of fluorescence in viable pollen has also been used to assess the quality of pollen (Heslop-Harrison and Heslop-Harrison 1970). Pollen

germination experiments are conducted by allowing the pollen to germinate in a pollen germination media. The emergence of a pollen tube from the pollen grain is then recorded as evidence of pollen viability (Wilson and Hoch 2009).

*Cytogenetic tests:* Polyploidy levels can be detected by chromosomal staining during cell division or by using more recent techniques, such as flow cytometry (Wilson and Hoch 2009). Individuals with odd ploidy or an abnormal cell division process are likely to be sterile or less fecund.

*Sterility genes:* Molecular markers linked to genes conferring sterility can be used to screen sterile cultivars. For example, marker-based (RAPD) selection techniques have been applied to facilitate rapid identification of male-sterile cultivars of peach (Jun et al. 2004).

*Common garden experiments:* Common garden experiments have been used frequently to assess the fecundity of sterile cultivars. Common garden experiments are often coupled with pollination experiments to determine the stability of the sterile cultivars after outcrossing (Spies and du Plessis 1987; Lehrer et al. 2006). Although common garden experiments are crucial for any evaluation procedure, they are time-consuming, and studies confirming long-term sterility are often lacking.

*Demographic models:* Demographic models are used to estimate the growth rate of populations using data about various life-history stages (Easterling et al. 2000). Data collected from experiments and natural populations can be effectively coupled with demographic models, such as population matrix models and integral projection models to predict population growth rates under different scenarios (Easterling et al. 2000; Geerts 2011; Knight et al. 2011). A population with a negative growth rate might be considered safe for cultivation. However, such models and their parameterisation are often highly context-dependent and caution should be taken when extrapolating results to different habitats or climates.

## Conclusions and recommendations

In this paper, we attempted to clarify the issue of distinguishing “safe” cultivars from “risky” relatives by recasting the problem as a set of six questions that align with the risk analysis process (Fig. 1). None of the individual questions is new; however, we hope that this formalisation will be valuable in providing an integrative framework for considering risks of infra- and inter-specific taxa. Although we focussed on ornamental plants, we believe that the set of questions can be extended to other situations (e.g., to breeds of animals), noting there will likely be additional ethical and cultural concerns.

While this set of six questions is, we believe, a useful formulation of the problem, answering the questions remains non-trivial. We highlighted the biological bases of reduced fecundity and sterility, and methods used to achieve and demonstrate this. However, there are many exceptions to each of the mechanisms and situations where particular methods do not work. In many cases, an unequivocal demonstration of sterility, and that any such sterility is stable, requires expensive and long-term field and molecular experiments. Various short-cut proxies of sterility have been proposed. For

example, the risk of different *Anigozanthos* spp. cultivars hybridising is a function of the ratio of their genome sizes; therefore, genetic exchange between horticultural and invasive populations can be limited if only taxa with sufficiently different genome sizes are allowed to be planted (Le Roux et al. 2010). However, long-term experiments are often necessary, specifically for woody and perennial species, before any conclusive evidence can be drawn about their invasiveness. Thus, the problem of trying to differentiate “safe” cultivars from “risky” relatives remains.

We hope the six questions outlined here will provide regulators with a basic structure around which a regulatory framework or protocol can be built and provide the horticultural industry with clarity over what needs to be demonstrated if invasions are to be avoided. However, given that the risks of invasion and impact are known from the “risky” relative, we conclude that the precautionary principle should be applied if unwanted consequences are to be avoided. We strongly believe that an *a priori* assumption of risk should be inherited from the closely-related invasive taxa from which the proposed “safe” alternatives are derived. This implies that the onus (and cost) of proof should be held by those who wish to benefit from infra- or inter-specific genetic entities.

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## Supplementary material I

### Table S1. Plant taxa listed under South African regulations for which certain sub-specific entities are listed differently from other entities

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Data type: species data

Explanation note: Plant taxa listed under the South African National Environmental Management: Biodiversity Act, Alien and Invasive Species Regulations as amended in 2016, for which certain sub-specific entities are listed differently from other entities. There is no published account as to why these taxa were selected.

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