The biosecurity risks of seeds in a botanic garden context

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Abstract

Seeds present a naturally occurring package of germplasm with ideal attributes for collection, distribution and, in the case of orthodox seed, long-term storage. From a phytosanitary perspective, seeds are often considered a relatively low-risk option for movement of germplasm across borders. Most published data are concerned with diseases of commercial crops and little is known about the risks associated with wild-collected, non-commercial seeds. However, there is demonstrable risk associated with the movement of any plant germplasm which can, in turn, pose a risk to both crops and the wider environment. Presented here is a discussion on seed legislation, standards and the difference between seed-borne and seed-transmitted pathogens, with case studies highlighting the risks associated with informal seed systems and wild-collected seeds in particular. Additionally, suggestions on how to address phytosanitary issues are presented, including awareness-raising measures aimed at improving biosecurity procedures during collection and before long-term storage of seed accessions.

Introduction

Most horticulturists view seeds as naturally occurring, convenient packages for the movement of 'starter material', i.e. plentiful, small, robust and, in the case of orthodox seed, offering good 'shelf life'. These attributes make seed an invaluable option for capturing genetic diversity for both *ex situ* conservation and breeding for preferred traits (Smith *et al.*, 2011). Seeds are assumed by many professional horticulturists and amateur gardeners to be the safest option from a plant health standpoint. This may be, at least in part, because historically most seed in the UK and EU was unregulated under plant health legislation. This changed recently and seed is now included alongside plants for planting. This change seems to be indicative of a more risk-based approach. Plant health legislation worldwide tends to focus on commodities in trade and enabling smooth flow of safe trade. Implementation of regulations can vary, especially when dealing with atypical material such as wild-collected seed and non-commercial plant taxa, and this may increase the risk of introducing new pests and pathogens.

However, seeds from any source – wild or cultivated – are far from risk free. In traded seed lots, provided infection levels are below a threshold estimated to be sufficiently low as to not pose a significant threat to the productivity and quality of any resulting crop, then seed will usually be distributed for cultivation. This system of tolerance levels for pathogens, which users are not always aware of, makes many assumptions about how the seed will be handled and any resulting crops

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grown, and any departures from these can have serious consequences for the grower and their crop (Roberts *et al.*, 1999). The presence of pests or pathogens in seeds may also be cryptic and it must be stressed that the presence of non-native organisms will not necessarily lead to the development of diseases in any resulting plants. However, it is crucial that any biosecurity risks associated with seed can be assessed and managed appropriately. There is more than a century's evidence to show that seed can be a significant pathway for the introduction of new plant pests and pathogens to an area, regardless of whether the seed was commercially produced or collected from the wild (Cleary *et al.*, 2019).

From the black rot bacteria (*Xanthomonas campestris* pv. *campestris*) of brassica crops (Shekhawat *et al.*, 1982) and tomato brown rugose fruit virus (ToBRFV) on tomatoes (Davino *et al.*, 2020; Dombrovsky & Smith, 2017) to *Megastigmus* sp. seed chalcids (wasp) associated with wild-origin native and exotic *Rosa* species growing at Royal Botanic Gardens (RBG), Kew (UK) and the Muséum National d'Histoire Naturelle (MNHN) in Paris (Roques & Auger-Rozenberg, 2016), a growing body of evidence demonstrates very clearly that seed from any source has the potential to act as an important pathway for the introduction of pests and pathogens to new areas. Botanic garden staff involved in biosecurity have been raising concerns about the potential for seeds from trade and botanic gardens to act as a pathway for the distribution of non-native organisms, including plant pests and pathogens (Symes, 2011). A recent study by Franić *et al.* (2019) screened traded tree seed lots of conifers and angiosperms and found that c. 30 per cent contained insect larvae. Fungi were isolated from 96 per cent of seed lots of which between 30 and 50 per cent were potentially pathogenic (in conifers and angiosperms respectively).

One of the 'unseen' risks is presented by viruses. These pathogens require a living host cell to replicate and are therefore intrinsically linked to the lifecycle of their host. Local dissemination of plant-infecting viruses is generally by means of a vector such as an insect (e.g. aphid, whitefly or thrips), nematode or fungal spore. Many plant viruses are also contact transmissible, or transmissible through root exudates and into water courses. Long-distance movement of plant viruses may occur via insects, however viruses can also be moved through plant propagating material. The intrinsic link between the virus and its host means that any vegetative plant part could carry infection, and the act of planting that material in a new region would allow the virus to establish. Through human activity, primarily international trade, multiple plant viruses have been moved around the globe into new regions, and in some cases these have caused economic or ecological damage (Van Brunschot *et al.*, 2014; Wylie *et al.*, 2014). However, any activity which moves potentially infected plant material across international borders carries a risk of bringing infection with it, hence the need for effective biosecurity measures to identify and mitigate against the risk of emerging diseases (Rodoni, 2009).

Here we discuss what we mean by 'seed' from a plant health perspective, and what measures are currently taken to limit the spread of pests and pathogens by formalised plant health regimes. Case studies will be examined where viruses have been intercepted in seed lots outside formal trade pathways, together with the implications these cases have for botanic gardens, plant

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collections and seed banks. Additional measures which could be implemented for improving procedures to enhance biosecurity at a local level are discussed.

Seed standards and legislation

The *Oxford English Dictionary* definition of seed is 'The unit of reproduction of a flowering plant, capable of developing into another such plant' (OED, 2021). Most horticulturists think of seed strictly as the result of sexual reproduction, with an embryo and food source or endosperm protected by a seed coat – the testa – encased in a fruiting body or drupe, or a pod in the case of legumes. In many agricultural systems, seed may also be the vegetative parts of plants such as tubers or seed stems.

Because pathogen lifecycles, especially viruses, are intrinsically linked to vegetative plant parts, vegetative reproduction strategies, such as the movement of plants for planting, cuttings and tubers, are recognised as having a higher potential for transmission and dissemination of viruses through regional and international trade. Where these crops have been bred into commercial production systems such as potato (*Solanum tuberosum*) or ornamental bulbs, the risk of these 'seeds' acting as sources of onward transmission is managed through the application of international standards for marketing and commercial quality control (Bianchi *et al.*, 2007; Grousset & Smith, 1998). In turn these standards are adopted into national and regional standards through official inspection and certification schemes. There are similar schemes for bulb crops; however, such schemes are costly, and in the developing world these are largely absent for food security crops such as cassava (*Manihot esculenta*). Commercially traded true seeds also have several regulations governing the minimum standards with which they should comply. However, for many seeds these standards are minimal, and for wild-collected seeds there are currently no recognised standards to ensure good biosecurity practices are observed during collection or onward propagation.

Legislation

Globally, plant health systems are guided by a series of standards implemented under the International Plant Protection Convention, which aims to 'secure coordinated, effective action to prevent and to control the introduction and spread of pests of plants and plant products'. These are known as the International Standards on Phytosanitary Measures (ISPMs). These standards are then used to inform the drafting of plant health legislation. The aim of plant health legislation is to prevent the importation of pests and pathogens which present the most significant risks to agriculture, horticulture and the environment, termed 'quarantine pests'. Additionally, there are controls on pests and pathogens which could severely limit plant production and trade if introduced or allowed to spread to their maximum extent, termed 'regulated non-quarantine pests'. In a botanic garden context, the movement of some seed within the UK and from UK-based sources requires a plant passport (PP). Even if the seed is listed as requiring a PP it may be exempt, depending on its intended use. The import of some seed from outside Great Britain is now subject to plant health regulation and requires a phytosanitary certificate (PC) and pre-notification of UK authorities as a minimum.[3] Some high-risk

[3] https://www.legislation.gov.uk/uksi/2020/1527/schedule/10/made

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taxa also require an inspection and further diagnostic tests on arrival in the UK, whilst plant genera considered to be very high risk, such as tuber-forming Solanaceae, may be prohibited from entry and can only be imported directly to licensed quarantine facilities. Wild-collected seed cannot meet the conditions required for a valid PC to be issued by exporting countries (e.g. health status of mother plant and inspection in the growing season) and should therefore be imported into the UK under a Plant Health Licence or a Scientific Licence, using a Letter of Authority (LoA), directly to a biosecure facility with the appropriate quarantine licence, as described above. Whilst it may prove possible to obtain a PC, it is a legal document which should be issued in good faith, and failure to do so may pose a risk to the relationship between the importing and exporting countries. It is key to note that plant health legislation is subject to change and National Plant Protection Organisations (NPPOs) can bring in specific restrictions to mitigate against emerging risks.

Currently, Northern Ireland (NI) is treated as a part of the EU Plant Health Regime whereas Great Britain (GB: England, Wales and Scotland) is separate from this legislation. All countries outside GB are now considered to be third countries. There are different rules and processes in place for EU and non-EU third countries.

Plant health legislation can be confusing to understand and implement, particularly when dealing with material which is atypical. This is partly because legislation has been developed primarily to expedite the movement of traded goods, including plants and seeds. The UK is no longer part of the EU, but EU legislation as it applied to the UK on 31 December 2020 has become part of UK domestic legislation and is subject to amendment. UK legislation can be accessed via the UK Plant Health Information Portal.[4] Whilst there is published guidance about UK plant health controls for imported material, it is a highly condensed summary aimed mostly at trade, which represents the bulk of plant imports. Seeking confirmation of GB import requirements with the appropriate authority (the Animal and Plant Health Agency (APHA) or Science and Advice for Scottish Agriculture) is recommended.

As of 14 December 2019, when major changes to UK plant health legislation came into force, other than *Vitis* and *Solanum tuberosum* seed from third countries which is prohibited, all other seed for planting imported to GB from third countries is described in guidance issued by the UK's Department for Environment, Food and Rural Affairs (Defra) as being either regulated, or regulated and notifiable. Each of these categories has different requirements for import (see Table 1). Regulated material must be accompanied by a PC and may require advance notification once a customs declaration has been made. Regulated and notifiable seed requires a PC and must be declared in advance of arrival in the UK so it can be inspected as it enters the country. It should be remembered that a PC is not a guarantee that material is free from pests and pathogens. Most PCs are issued on the basis of a single visual inspection, so a PC may be issued in good faith when pests or pathogens present in seed or plant material may not be apparent. The onus is on the importer or recipient to manage the material – and the risks it may pose – appropriately.

When an organisation wishes to import, move or keep material that is usually prohibited (including plants, parts of plants,

[4] https://planthealthportal.defra.gov.uk/

Table 1 Overview of requirements relating to import of seed material to Great Britain.

Category of seed material	Regulated	Regulated and notifiable	Regulated or Regulated and notifiable	Prohibited
	Where a valid PC can be issued	Where a valid PC can be issued	If conditions mean a PC cannot be obtained	
Documentation required for import to GB	PC	PC	Letter of authority (LoA)	LoA
Containment requirements	No statutory quarantine necessary	No statutory quarantine necessary	Compulsory quarantine	Compulsory quarantine
Can plants grown from this seed be released from quarantine/ licence terms?	N/A	N/A	Yes	Potentially, although it may not be practical or affordable
Requirements for release	N/A	N/A	Inspection post-entry by a Plant Health and Seeds Inspector (PHSI) followed by a minimum of 12 months' quarantine, following germination. For material where specific conditions must be met for release then successful test results will be required. If plants are found to be free from injurious pests and pathogens, on inspection by a PHSI, the material will be issued a quarantine release certificate, releasing it from the terms of the licence.	Release from quarantine conditions for prohibited material (in accordance with Regulation 2019/2148) requires implementation of a species-specific quarantine release protocol and terms must be agreed in advance with Defra and the APHA. Some tests may take up to two years to complete. All costs are borne by the importer of the material.

seeds, fungi, algae, plant pests, pathogens or soil) or which is regulated and cannot be issued with a valid PC then a Scientific Licence or a Plant Health Licence will be needed. These licences are available only for a limited range of uses such as official testing or scientific research. Use of these licences requires appropriate facilities including plant quarantine and standard operating procedures to be in place. Licensed material is imported under an LoA and may be shared or exchanged with other licensed facilities using appropriate written authorisation: notification form PHI10 (a request for authorisation to send material to another licensed facility) and an LoA.

Seed standards

Seed can be produced through a deliberate and controlled process of production (commercial seed), by saving part of a managed commercial crop (farm-saved seed), from a garden (home-saved seed) or through collection from the wild (wild-collected or natural-source seed). In the UK many types of seed marketed commercially are subject to legislation which requires the seed to meet certain minimum standards of genetic conformity (i.e. is it true to type?), physical purity (absence of chaff, weed seeds, soil, debris, etc.), and vigour and viability.

Commercial seed production is divided into several categories, generally denoting an increase in bulking stages and field generations and consequently a loosening of thresholds for the presence of some diseases. The precursors of certified seed are nuclear/basic stocks, breeder seed, pre-basic, basic and then certified seed. It is certified seed that is grown to produce the 'crop' (which can be used as a source of farm-saved seed). The more cycles of seed generation that occur the larger the seed numbers involved and the lower the genetic purity of the seed crop, even if regular inspections and rogueing (removing plants which are unhealthy or not true to type) take place. Despite the high value of nuclear stocks most are not screened for diseases before being used to produce breeder seed. Certified seeds must be of known varieties on the UK national list or, in the EU, listed in the EU common catalogue, subject to the Seed Marketing Regulations 2011 and Technical Standards. In a wide range of species, such as beet, cereals, fodder plants, legumes, crucifers and fibre crops a licence from the APHA to sell, market, repack or process these is required, and the crops are subject to regular inspections, which may comprise visual inspections of the crop, test plots and seed, and accompanying diagnostic tests if required. Testing is often limited to ensuring seed is of a suitable quality in terms of purity, viability and germinability. Where seed health, in the strictest sense, is tested this is usually not about certifying the absence of a specific pest or pathogen, but to indicate that levels of disease are below a set threshold (i.e. a tolerance level) which could lead to unacceptable levels of losses in commercial crops. It is crucial that those importing and growing seeds, whether the seeds are cultivated or of wild origin, are aware of the risks associated with them and manage them appropriately.

Non-commercial seed production ranges from the production of garden or 'farm-saved' seed to seed-sharing initiatives and cooperative breeding programmes. In the case of botanic gardens and arboreta, non-certified seeds of species of interest are obtained through inter-institution exchange, from seed banks or botanic garden seed lists (*indices semini*). This can include wild-collected or 'natural-source' seed obtained through fieldwork by botanic garden staff or colleagues in country. Given that an estimated two in five plant species are under threat of extinction (Antonelli *et al.*, 2020), the focus has understandably been on collecting and conserving as much wild plant diversity *ex situ* as possible, often relying on extremely limited resources. Any biosecurity implications relating to this seed have been secondary – and, for some, unconsidered. The same cannot be said about other types of invasive species, for instance.

Invasive non-native or alien species – including plants, animals, fungi and microorganisms – are recognised as one of the main drivers for global biodiversity loss.

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The impact of invasive non-native species on biodiversity and the importance of preventing their introduction, establishment and spread to new ranges is recognised and accepted as requiring both political and practical effort and resources in the Convention on Biological Diversity (CBD) Article 8h where contracting parties commit to 'prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species'. Botanic gardens have also made a collective commitment in the Global Strategy for Plant Conservation, aiming to implement the biodiversity targets of the CBD. Objective II aims to 'ensure that plant diversity is urgently and effectively conserved', Target 8 that 'at least 75% of threatened plant species [are] in *ex situ* collections, preferably in the country of origin, and at least 20% [are] available for recovery and restoration programmes' and Target 10 that 'effective management plans [are] in place to prevent new biological invasions and to manage important areas for plant diversity that are invaded' (CBD, 2020). Given that in the UK the Non-Native Species Secretariat reports that 75 per cent of non-native species introduced are plants, 22 per cent invertebrates (largely insects) and the remaining 3 per cent mammals and other organisms (GBNNS, 2015), it is perhaps unsurprising that botanic gardens have focused their efforts where their expertise and interest lie – plants. As understanding and awareness of the biosecurity risks associated with seeds increase, there is a need for the sector to develop and communicate the tools and expertise required to address the challenge of collecting, growing and sharing seeds safely so that the needs of plant conservation and research can be delivered whilst addressing biosecurity risks.

Seed-borne vs seed transmission

As previously discussed, vegetative propagative material such as seed tubers, bulbs and stems provide a living host that can allow viruses to bridge between seasons. Viruses transmitted via this mechanism will establish with planting of their host material, allowing a clockwork infection-establishment relationship. However, this is not the same with viruses transmitted via true seeds. For true seeds there are broadly two mechanisms involved in seed transmission of virus:

1. seed-borne infections, with the virus carried as seed coat contamination infecting the emerging seedling
2. the virus being carried in the plant tissues of the embryo (Hull, 2013).

Due to the nature of seed-borne infections, transmission rates tend to be low, effectively at trace levels; however, in commercial planting systems these low rates of transmission can still lead to devastating outbreaks. Consequently, there are biosecurity protocols which can be applied to mitigate against the risks of seed-borne infection, such as chemical or heat-based disinfection strategies (Davino *et al.*, 2020; Sauer & Burroughs, 1986). Whilst these approaches can be effective, they may not always be enough to fully eliminate infectivity (Reingold *et al.*, 2015). Another confounding factor is that the molecular diagnostics methods used for a lot of border biosecurity assurance testing may still detect the presence of nucleic acids (DNA and RNA) even after they have been denatured (Davino *et al.*, 2020). This factor may still lead to phytosanitary action being taken in these situations.

The rate of transmission for seed-transmitted viruses can vary greatly and is

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influenced by several key factors including the species and strain of virus; the host plant and variety; the timing of infection; the growing conditions; and the age of the seed. In cases of seed transmission via an infected embryo, control presents a challenge, though heat treatment can be effective for some viruses without adversely affecting seed germination.

The phytosanitary risks associated with seeds

Some international standard diagnostic protocols covering seed-transmitted viruses have been issued by the International Seed Testing Association and the International Seed Federation, but these are not comprehensive. They often use old technological approaches such as grow-out tests and biological inoculation assays, due to concerns within commercial seed production of the detection of 'inactive' virus. However these biological tests can take several weeks to complete and are not suitable for routine testing of seed in transit such as biosecurity import compliance screening. The range of diagnostic methods available for seed testing is as broad as those available for any other pathogen including serological (ELISA) and molecular (PCR and real-time PCR) techniques. These diagnostic advances have been comprehensively discussed by Boonham *et al.* (2014) and others. However, the complications posed by validating testing for asymptomatic screening mean that developments in seed diagnostics track behind other diagnostic applications of these technologies. Even the latest non-targeted technologies such as high throughput sequencing (HTS) have been investigated for seed testing applications, but these have yet to be adopted into routine testing (Fox *et al.*, 2015).

Plant health biosecurity and plant health regulation rely on species listings (Jones & Baker, 2007). The limitations of these have been discussed from the perspective of advancing diagnostics technologies (Adams *et al.*, 2018b; MacDiarmid *et al.*, 2013), however there are also limitations in terms of highlighting potentially high-risk plant species. Where this is most apparent is in the ornamental species which are traded in large volumes and across a vast range of plant species and cultivars. The listing of high-risk pests also has limitations in being able to rapidly respond to newly described emerging transboundary plant pests or pathogens, as these may be widely distributed before there is broad awareness of the risk presented by them.

Throughout the 1990s and 2000s the increased trade in ornamental plants, especially bulb crops, allied to improved diagnostic technologies led to a marked increase in first virus records for the UK (Fox & Mumford, 2017). Similarly, there are a broad range of niche crops which may not be fully characterised in terms of associated risks and consequently do not appear on regulatory lists: either heritage crops such as skirret (*Sium sisarum*) or exotic crop species unsuitable for commercial production in the UK such as mashua (*Tropaeolum tuberosum*) and oca (*Oxalis tuberosa*) which have an interest for the specialist or hobby grower.

Another area where such regulatory lists may not cover the breadth of potential risks is in crop-wild relatives either for growing as ornamental species or as part of conservation collections. The case studies below give examples highlighting the biosecurity risks associated with both vegetative seed tubers and true seeds.

Case study: emerging risks associated with commercial seed trade

Commercial seed of species associated with seed-transmissible quarantine pathogens is routinely tested during trade; however, even with these rigorous checks, emerging seed-transmissible diseases can rapidly escalate into epidemics (Jones, 2021). Examples of this are ToBRFV and tomato mottle mosaic virus (ToMMV). These are both members of the genus *Tobamovirus* and are robust, contact-transmissible viruses which can cause severe economic damage to protected crops such as tomato (*Solanum lycopersicum*) and pepper (*Capsicum* spp.). ToMMV was first reported from Mexico in 2013 (Li *et al.*, 2013), while ToBRFV was first reported from Jordan in 2016 (Salem *et al.*, 2016). Tobamoviruses are recognised as seed-transmitted pathogens, and this mechanism has been demonstrated for ToBRFV (Davino *et al.*, 2020; Dombrovsky & Smith, 2017). Both viruses have become transboundary plant pests, although ToBRFV has attracted more attention due to its greater distribution and consequent impact. The virus has now been reported from most European tomato-growing regions as well as the USA, Mexico and China (EPPO/OEPP, 2021b).

Due to the risks associated with outbreaks, applied research has focused on detection in commercial crops and seed lots, and mitigating spread of ToBRFV through seed disinfection (Davino *et al.*, 2020; EPPO/OEPP, 2021a; Samarah *et al.*, 2021). Currently, third-country seed of susceptible hosts entering the UK, the EU and many other countries is tested for the presence of the virus. The global spread of the virus appears to be slowing, with fewer new countries reporting outbreaks in 2020 than in preceding years. This is likely to be a combined effect of industry awareness and statutory import testing. The approaches taken to mitigate the potential spread of these viruses through testing and prophylactic disinfection are steps which could be readily adapted and applied on a routine basis during seed collecting for botanic gardens and would reduce exposure to potential outbreaks of pests and diseases.

Case study: the risks from niche crops

Ulluco (*Ullucus tuberosus*) is a tuber-forming species in the family Basellaceae. It originated in South America and is found in Argentina, Bolivia, Chile, Colombia, Ecuador, Peru and Venezuela. The vividly coloured tubers are edible and widely consumed in the Andean region, but there has been a growing interest in ulluco from hobby and specialist growers. In 2017, the UK NPPO identified ulluco plants being grown for sale as seed tubers in contravention of seed production regulations. The plants exhibited a range of symptoms consistent with viral infection, such as yellowing, reddening, leaf crinkle, dwarfing, spotting and leaf deformation. Samples were submitted to Fera Science for testing, and these were screened for the presence of known listed quarantine pathogens which had been previously reported as infecting the species (Brunt *et al.*, 1982; Lizárraga *et al.*, 1996a; Lizárraga *et al.*, 1996b). The initial screening indicated the presence of viruses at high incidence, often in mixed infections, including Andean potato latent virus (APLV) – a high-risk quarantine pathogen. However, the identity of this virus could not be confirmed, and HTS analysis indicated that the viruses detected were new to science but related to quarantine-listed pathogens (Fox *et al.*, 2019). Preliminary biological characterisation of these novel viruses indicated that they may pose a risk to solanaceous species such as tomato. As a consequence, ulluco was added to the EU list of high-risk plant species.

Further investigations revealed several tuber species being sold via the internet and shipped internationally. Tubers of mashua and oca were also found to be infected with multiple novel viruses, including some from genera which may be transmissible through true seed. These viruses were not thought to present a high plant health risk, and work is ongoing to biologically and molecularly characterise these viruses (Adams *et al.*, 2018a). The number and range of viruses detected indicate poor production hygiene and the transmission of these viruses through the production system. More recently, tubers of yacon (*Smallanthus sonchifolius*) were found to be infected with potato yellowing virus, another high-risk quarantine virus (Silvestre *et al.*, 2020). This virus belongs to a genus where most member species are known to be transmissible through both seed and pollen.

Case study: the risks from wild-collected seeds

Sourcing plants and seeds from wild-grown species is common practice in botanic gardens, especially those species of significance for conservation and biodiversity collections. During collection, however, biosecurity and hygiene best practices for disease management do not appear to be commonly considered. The presence of diseases stored within germplasm collections have previously been identified as presenting a risk for future crops (O'Hanlon *et al.*, 2019). Botanic gardens play a crucial role in maintaining biodiversity and as sources for diverse plant germplasm for future plant breeding to support plant resilience (Heywood, 2011). For example, the Millennium Seed Bank (MSB) at RBG, Kew is a custodian of collections covering around 37,000 plant species, around 74 per cent of which are endemic, endangered (nationally or globally) and/or have an economic, ecological, social, cultural or scientific value. Around 10 per cent of these species are listed as vulnerable, rare or extinct. In 2018 it was reported that over 11,000 seed samples had been distributed globally for conservation, research or education (Liu *et al.*, 2018), underpinning this key role as a global resource. One area where these collections provide obvious value is in the provision of germplasm from crop-wild relatives which can be screened for desirable traits to introduce for potential crop improvement via breeding activity (Dempewolf *et al.*, 2017).

During 2018 seed samples of a range of non-cultivated taxa related to *Solanum melongena* (aubergine) were being transferred from the MSB to the World Vegetable Centre in Taiwan. These had been collected as part of the Adapting Agriculture to Climate Change (Crop Wild Relatives) project. To comply with phytosanitary certification, seed samples were tested for the presence of potato spindle tuber viroid (PSTVd), a robust, seed-borne, virus-like pathogen. Of ninety-eight seed samples tested, eight were found to be infected with PSTVd including *S. anguivi*, *S. dasyphyllum* and *S. coagulans* – all new host records for the pathogen. Utilising herbarium samples collected contemporaneously with the seed samples, the findings from Uganda and Kenya were used to confirm both the species and country records for these detections, representing the first records of PSTVd in East Africa (Skelton *et al.*, 2019). These findings have implications for food production, food security and breeding of solanaceous crops in the region.

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The way ahead…

Raise awareness

Among horticultural professionals there are varying levels of awareness and understanding of the biosecurity risks posed by seeds and, in particular, wild-collected seeds. This stems partly, it seems, from a belief that wild species are less susceptible to pathogens than cultivated species, even though relatively little work has been carried out on the role of wild species, especially as a part of the broader disease pathosystem.

Given the increasing interest in and importance of horticulture generally – from wellbeing benefits to food security, *ex situ* conservation and landscape restoration – it is vital that those managing and working with seed understand the associated risks and avoid making assumptions. Where pathogens occur, they may be inactive and whilst this is currently interpreted as posing no 'risk' to the current host this may not continue to be the case, particularly when the seed biome is subjected to other biological and environmental factors on sowing. It also cannot be assumed that because one taxon is currently unaffected by a pathogen, there is no risk to other potential hosts. This is borne out by the experience of the authors, where known host ranges for many plant pests and pathogens have been shown to be considerably wider than previously recorded when these organisms have been identified in botanic garden collections – often despite presenting no clear symptoms to indicate their presence.

Even within the professional horticultural community, the danger posed by seed-borne pathogens is greatly underestimated, and so the need to raise awareness of the associated risks should be considered a priority. From a general public perspective, the current 'Don't Risk It' campaign is designed to raise awareness of the risk of bringing plant material back from holidays and overseas trips (Brunel, 2014). However, there is little awareness of the variable biosecurity risks associated with seeds from different sources such as breeder seed, farm or garden-saved seed, and wild-collected seed. A broad drive to disseminate this message and engage the general public, utilising diverse organisations such as the Royal Horticultural Society and botanic gardens, focused on major public events such as 'Plant Health Week' or the Chelsea Flower Show, would increase awareness of the compelling arguments for acquiring certified seeds from a reliable source.

Revised approaches

The general approach to seed collection, management and use needs to be updated to improve risk management and uncertainty. Biosecurity best practice protocols and the precautionary principle should be guiding concepts when collecting and working with seed in any context. This is especially true when the seed is of wild origin. In this way the risk of introducing new plant pests or pathogens to a plant collection, landscape, country or continent can be reduced.

Seed should always be collected with care, selected from mother plants that – at least from outward appearance – are healthy and free of pests. Collection of seeds and seedbearing structures from the ground should be avoided wherever possible, as this increases the likelihood of contamination, particularly by insect pests and soil-borne micro-organisms. Seed should be managed to maximise health and vigour, and hygiene best practice should be followed from seed collection through processing and storage to end use.

Ideally seed should be:

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- tested for the presence of pests and pathogens prior to storage or use:
  - where resources permit, X-raying for pests – otherwise, cut-test a subsample or 'incubate' seed batch in closed containers at elevated temperatures to encourage emergence of insect larvae or adults
  - in the case of pathogens, possibly through bulk testing a wash from an aggregate sample prior to sterilisation, allowing non-destructive screening
- nursery raised under quarantine conditions, including:
  - seed sterilisation
  - separation of taxa – growing species with each separated by a cordon sanitaire
  - where possible, station sowing of seed to ensure individual seedlings are isolated from their neighbour, to avoid cross-contamination in the event that one is infected
  - good nursery hygiene practices – sterile containers, fresh compost, clean tools and hands, clear and accurate labelling and record-keeping
  - controlled watering by avoiding overhead irrigation and containing run-off
  - appropriate climate conditions with good airflow
  - appropriate disposal of plant waste and spent compost
  - routine monitoring and removal of infected or unhealthy plants (rogueing)
  - regular inspection and testing of seeds and plants prior to incorporation in collections or planting in the landscape, whether for *ex situ* or *in situ* conservation

In the future, routine testing of all wild-collected seed would be desirable; however, implementing this type of approach would be challenging for practical, logistical and, in some cases, ethical reasons. Broad-based testing could be prohibitively expensive. Additionally, confirming the breadth of testing required would also present a challenge due to the lack of information available for many wild-collected plant species. Whilst using an approach like HTS may seem to offer a solution to the question of what to test for, the potential for such an approach to detect an array of incidental, previously identified pathogens could present barriers to legitimate seed dissemination where the associated risks are minimal. Therefore, one approach would be to limit any testing to the known regulated pathogens associated with the genus of plant in question. The other issue with wild-collected seeds is their high cultural and scientific value, often exacerbated by the low volume of seeds collected for each accession. Seed testing is traditionally a destructive process which is not ideal for low-volume, high-value seeds. This could be mitigated against by targeting genera tested to those related to high-value crops or high-risk taxa such as Poaceae, Solanaceae, Rosaceae, Apiaceae, Alliaceae and Fagaceae, and limiting destructive testing to a random selection from collected samples. Another alternative would be to further investigate the applicability of non-destructive testing methods, such as those developed for commercial tomato seed screening through AHDB project PC229 'Wash and Grow'. This approach allows seeds to be returned to storage after testing as it is non-destructive and could be explored and validated for use in screening high-value material. The other consideration involves the value and

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ownership of the biological data generated through testing. Sequencing data, also termed digital sequence information (DSI), is currently under consideration for inclusion under the Nagoya Protocol on access and benefit sharing of the CBD (Buck & Hamilton, 2011). However, at the time of writing (2021) this discussion is ongoing with competing viewpoints as to the value and impacts of the inclusion of DSI within the protocol (Ambler *et al.*, 2020; Karger & Scholz, 2021).

There is also a need for further work into the prevalence and risk posed by pathogens associated with wild-collected seeds. Currently little is known about the role of crop-wild relatives as either alternate or reservoir hosts. However, the report from Skelton *et al.* (2019) found evidence of PSTVd in nearly 10 per cent of seed lots tested, suggesting, for some genera at least, that the biosecurity risks associated with wild-collected, crop-wild relatives may be underestimated. There is a need to better understand this risk and the broader role of such hosts in pathogen infection cycles. Whilst broad baseline surveillance would be desirable this may be best achieved through partnership working in the countries of origin of the collected material. However, as demonstrated through the Skelton *et al.* (2019) report, the potential value of untapped baseline data in seed lots which have already been collected and stored should not be underestimated.

Conclusion

The commercial seed trade is regulated and, although risks such as that highlighted with ToBRFV are present, there are both visual and diagnostic checks during production and in trade to mitigate against the risks of importing seed with damaging viruses or other pathogens. With exotic and unusual plant species these checks are more challenging due to the lack of prior knowledge of associated pests and diseases. With wild-collected seeds these production checks prior to harvest or collection cannot be easily carried out. Therefore it is important to raise awareness of the potential risks associated with seed of many genera. This needs to be combined with implementation of rigorous hygiene and diagnostic strategies to mitigate against inadvertent introduction of pests and diseases. High-throughput sequencing may offer a non-targeted diagnostic solution to allow screening for pathogens without *a priori* knowledge of associated pathogens. Not all 'agents' which might be detected are necessarily pathogens, however, and work is needed to understand the roles of all members of the microbiome associated with seeds of a given species. A project that will assess the diversity, function and risks associated with the microbiome of wild-collected seeds has just been commissioned by Defra, with collaborators from Fera Science, Forest Research, RBG, Kew, and Botanic Gardens Conservation International. The aim of this project is to understand the risks of seed-associated pathogens and to generate guidelines and mitigation measures to minimise the risks from wild-collected seeds.

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