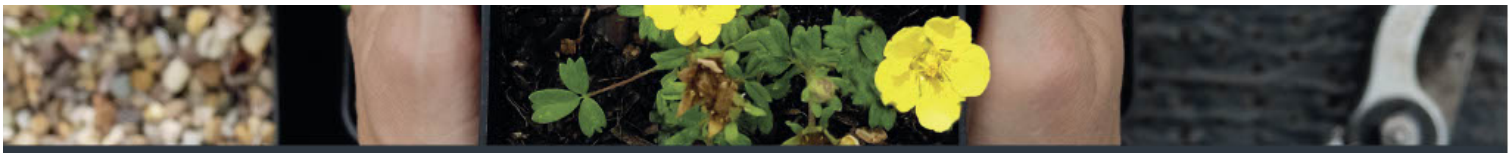


Promoting Excellence in Horticulture

Transcript for Parallel Session 1: ‘Growing from seed: the next frontier in biosecurity’

Speaker 1: Adrian Fox

Good afternoon, my name is Adrian Fox. I'm the senior virologist at FERA Science in York. And for this session, which we've titled growing from seed, the next frontier in biosecurity, I just want to cover some of the issues around viruses in seed and the risk to plant health, that bringing in seed could actually pose in the future. So I think it's probably more accurate that we, we call this session growing viruses from seed, the next frontier in biosecurity.

We'll start by just talking a little bit about what we define as seed. Now if you're a little bit exposed to talking to botanists about what is and isn't seed, but just from a plant health sense, and as a plant virologist, all the different things that we could consider as a seed or input material. And I'll also talk a little bit about the different diagnostic developments that have gone on certainly over the last 10 to 15 years, and how the diagnostic technologies that we now have are impacting on our understanding of plant health. And that we're finding a lot of things that even five years ago, we didn't even know existed, or we're finding things in new hosts. And I'll cover all of that as we go. But really, the main bulk of the talk will be some case studies on on the risks of seed in a Botanic Garden context. So I'll talk a little bit about niche crops. And that will then lead into a case study on regulated trades. And I'll finish with a case study on the risks of wild collected material. And just by way of summary, at the end, there's a slide on, on how I think we can move forward together to both ensure good plant health, but also to allow botanic gardens to to act in a responsible way and make sure that they are as bio secure as they can be.

For the purpose of this talk, I think it's key to define what we mean by seed and I would class seed as being anything that is used as an input material for growing a plant. So we all think of a true botanical seed. So here we've got the picture of tomato seeds to represent that. But you've also got things like leguminous seeds, so the peas, but also also consider things like seed potatoes, and other tubers, so the picture of the nice colourful heuchera in the top right. But also we can consider things like vegetative stem cuttings, such as those used in cassava, which are also described as a seed chain. And there's a different level of biosecurity risk applied to whether it's vegetative or true seed, obviously a vegetative input where a virus will automatically in establishing a new area or if it's brought in in the vegetative input material, that poses a higher risk. However, I think, traditionally true botanical seed has always been seen as being very low risk. However, there are viruses, fungus, and bacteria, which can all hitch a ride on that seed. And if if you don't look after the biosecurity in the cleanup procedures when importing, it can pose a biosecurity risk downstream from that. And I'll include case studies that cover both tuberous and the true botanical seed.

Plant virology as a discipline is about hundred and 50 years old now. But as a discipline, we've come a long way from from passing infected sap through through a Chamberlin filter, as you see on the left, and then infecting plants with with the sap that comes through the filter to show it's still infectious. And over the years, we've moved through biological testing, and we've moved through serological testing, such as the ELISA plates and the alternate double diffusion that you see.

And we move into the molecular methods. So things like the PCR, or real time PCR, and we've also dabbled with infield diagnostic techniques. And on the right, you can see the lateral flow devices. So similar to a pregnancy testing kit idea, where you have a specific antibody that will detect a virus or the pathogen, and you drop a bit of sap on to the the well and as it runs across, you get a possible negative. We've taken that one step further with the lamp of diagnostics that you see on the middle right. But the big the big step change in diagnostic technologies over the past 10 years certainly has been the developer

development of high throughput sequencing, and that's the blackened cream box that you can see on the bottom right hand side. And that is really a non targeted method. So the PCR and the analyzers that we haven't even that the lamp testing are all we'd call a targeted testing method and that allows us to detect specific, specific pathogens. So we would use an antiserin or a primer set for for a given virus. And that would allow us to detect that. But the high throughput sequencing takes us into a different realm of detection. It's a non targeted approach. And so what it allows you to do is detect any plant virus nucleic acids that may be present within a sample. And then you can look at the data that comes out of it and interpret exactly what virus is present. And that's really moved design and diagnostic techniques to to revolutionise some of our understanding of plant virology. And we're now flushing through new viruses, and we're finding viruses in hosts that we didn't know could could host the viruses.

So one of the first case studies is really the risk that can be posed by niche crops. And this kind of flows into our second case study as well, because most of what I'll talk about in these first two case studies are tuberous crops. And this goes back to the summer of 2017. When the animal and plant health agency were informed about a small hobby grower in the south of England, who was growing a Ullucus tuberosus, and they were selling this material as seed on via the internet. And that, obviously, is in contravention of plant health regulations if, if it doesn't have appropriate inspection, and the grower isn't certified. And so, when the inspector went to look at this material, he thought that it was showing signs of virus. So we seized the material got it brought up to Fera for testing. And our initial screen suggested that there were currently viruses present there. One of which was Andean potato latent virus, which is a potential infecting virus that's only been reported from South America. And so that would obviously be of great concern for plant health services. However, we couldn't confirm the the presence of that virus of any of the other potential quarantine viruses that we found by ELISA. So we turn to the high throughput sequencing this non targeted method. And what we actually found was that, through that genetic sequencing, we revealed six viruses that were new to science, and also non native pathogens, and of those certainly two novel viruses, we considered to be high risk, and that was because they were closely related to South American viruses that would infect the solanaceae. So if you look at this, this phylogenetic tree on the bottom right, everything above the purple dotted line has been reported from South America. And the two red boxes are around Andean potato latent virus, which is a one A one quarantine pathogen, and Andean potato mould was a virus which is on the apo alert list. And these two new viruses sit right in between those two viruses. Additionally, we also found a virus called papaya mosaic virus and the Ullucus-strain of the papaya mosaic virus is also recognised for tomato and on the basis of that plant health action was taken to the extent that Ullucus was actually included on the European legislation as a high risk plant species that couldn't be imported into the EU.

And so this led us on to our second case study, which was really about the risks that securing material over the internet poses. And so we went around a number of channels, a popular internet shopping sites, and we focused in on those Andean tubers. So ullucus was one of those but there's also mashua, oca and yacon. But we also found as we were going, some things that were listed as ornamental potato hybrids, which came as tubers. We bought some true potato seed, which originated in Russia, of all places. And we also bought some squash, some flax and some chilli pepper seed as well. And these were sourced from Croatia, the Netherlands, USA, UK, Poland and Ukraine. And the interesting thing is, when you start looking at the Andean tuber varieties that we bought, some of them had kind of typical names that you'd expect from, from South America, something like Cusco market and Bogota market, which may give a clue where this material originated from. But when you then start buying things that are called New from Peru, then you're really starting to ask questions about whether this is material that's been grown in Europe for many generations, or is material that's been imported from South America directly for shipping around in the European Union, and to the UK.

And interestingly, some of our some of our purchases didn't have cultvar names at all. They were just listed under numerical codes. And again, that would suggest that there's either some breeding material going on, or someone somewhere is raiding a gene bank for this material. Interestingly, there was a range of, shall we say, of, of levels of professionalism in the material that

we received. So this is some material received from the Netherlands which was well packaged, nice clean tubers. And with that when we got a free gift I will talk a bit more of a free gift in a moment. Some of it was not so well packaged, I think I'm not sure this would pass any any level of scrutiny. And again, just wrapped in newspaper and there you can see new from Peru, and as you see a range of tubers, and so when we put that stuff through the sequencer, the ullucus we just found the range of the viruses we'd already found. But we also found a novel tyro virus at a novel polara virus, which cross reacts with potato leaf roll and again would potentially be a quarantine risk. We found in mashua, we found candidiasis phytoplasma forgari, which is a pathogen that we'd like to take action on. And we found a novel virus, which we later found is the same as a virus called mashua virus y, which was originally tested and found described in 1984 from Peru. The greatest concern in this material, within yacon, we found a virus called potato yellowing virus. And this, again, is a South American potato infecting virus, and would be a cause for plant health concern. And interestingly, as we're doing this work, we also had a UK interception via a Botanic Garden of this material, and so it shows that these these niche crops, these crops of interest, which quite often make it into the kind of food gardens that quite a lot Botanic Gardens have, can actually be a present a real plant health risk.

And the final case study is really to talk about the risk that true seeds can present. And we know that some virus and viroids present a risk through seed transmission. And they're also present in wild and ornamental hosts. And so for those that the main concerns with things like the tobamum viruses, such as tobacco mosaic virus, or this virus that we see on the top right, which is to tomato brown virus, which is causing major problems with the tomato industry at the moment. And these remain active on common surfaces for long periods of time. They're transmitted by seed and through mechanical contact, but they're also heat stable. And then the other group of pathogens were particularly concerned about viroids, which are the smallest pathogens known. And again, they're transmitted by seed and through mechanical contact, and again, they remain stable. And both of these groups of viruses are seed transmitted. And the key thing to remember is wild hosts as well as ornamental hosts may be tolerant to these. So you don't see symptoms, but there can be infected and can pose an infection source for for surrounding plants.

So, back in 2018, we intercepted some some viroids in in seed, that were actually moving from a Botanic Garden collection for for breeding material. So as part of a project looking at crop wild relatives, there were about 30 wild taxa that were related to African eggplant that were collected from across Africa. And they were most important, there were no symptoms observed on collection of this material. And all this material was cleaned and dried in country. Then there was some further prep and storage when the material got to the Millennium Seed Bank. And then in 2018, nearly 100 of these seed samples were identified for pre breeding, and they were being sent to the World Vegetable Centre in Taiwan, and to meet import regulations, because these are a solanaceae, there was a requirement to show freedom from potato spindle tuber viroid. But because there was relatively little material to test, we agreed that we would only test 10 seeds from each seed sample. And from these, for we actually found eight samples positive. And each of these solanaceous species is actually a new host for for pstvd. And interesting. We also tested associated herbarium specimens, which was submitted for screening as part of our follow up investigation. So in terms of African outbreaks of PSTvd, well, we knew that they'd been outbreaks in potato in Egypt and there are records in tomato from Ghana and Nigeria. But we've now added these three new solanaceous species as new hosts on the basis of seed findings. And also we can confirm an extended distribution of Pstbd into East Africa whether no been reported before, given that we found material in herbarium specimens collected in Uganda and Kenya.

So how do we move forward from here and I think in terms of Botanic Garden work, don't assume that something is virus free just because it's asymptomatic. And while something's present that may not pose a risk to the current host, it may present a risk to other hosts. So if you're growing seeds from, shall we, shall we say, an unknown origin or where you don't have provenance for that seed. I think it's key to to implement a cordon sanitaire, and use good hygiene practices when growing out seeds. So treat them as a quarantine issue. And it would also be an idea to review collecting procedures to include good biosecurity protocols. So whether that's a cleanup set process within your your seed extraction, implementing routine testing

to check for, for risk viruses, and then also think about ways that you can minimise crossover in the field. Because if you're dealing with, shall we say, limited plant resources in the field, the last thing you want to do is cross contaminate an infected plant into healthy plants. And I think the other thing that would be really key from my perspective is given we know very little about the presence of viruses in non crops and wild relatives, there's a real need for baseline studies to include wild relatives within them. And that's something that I'd certainly be interested to do. And there are ufresco projects in the near future to look at baselining studies. Thank you for listening.

Speaker 2: Sara Redstone

Hello, everybody. My name is Sara redstone. And I'm the plant health and quarantine officer at the Royal Botanic Gardens Kew. I'm going to be talking to you over the next 10 or 15 minutes about growing from seed, the next frontier in biosecurity.

The Millennium Ecosystem Assessment estimates that approximately one quarter of all known plant species are threatened with extinction. This means that the ability to share seeds and the plants that we grow from them safely has never been more crucial. Over the next 10 to 15 minutes, we're going to look at some different types of seed, the legislation that applies to them, and consider internal and external contamination very briefly, we'll then look at some of the biosecurity risks and how we can mitigate them.

Seeds are a very convenient way to share plant material, offering maximum diversity for minimum size until recent changes to the plant health legislation, they were also largely free of any paperwork requirements. And perhaps for this reason, they have often in the past been assumed to be the safest option in biosecurity terms. We now know that this is very much not the case. If we think of the range of circumstances where we might use seeds and the plants that we grow from them, then it should be clear why it's important to ensure that that seed isn't carrying any newer exotic pests and pathogens. This is particularly the case when looking at landscape restoration and ecological restoration projects.

Seeds of many different types from many different sources are used in horticulture and the biosecurity risk varies significantly according to the origins of that seed. In the case of seed in seed banks in germ plasm banks, most is not tested for pathogens unless there is a specific need. Of course, when we are testing for pathogens, the question is what to test for? Do we test for the knowns or the unknown? If we test for unknowns and find unknowns, what do those results mean? It's not a straightforward question. As we'll see shortly, new regulations mean that more testing and documentation is required to import and export seeds.

Plant pests and diseases don't respect political or geographical boundaries. Seeds have been recognised for some time as a significant pathway for the introduction and dissemination of pests in new geographic areas through the seed trade. And because of this, the International Plant Protection Convention developed ispm 38 to control the international movement of seeds. This is one way that we address the issue of seed as a pathway for pests and pathogens.

Welcome changes to address biosecurity risks associated with the international movement of seeds came into force in the UK on the 14th of December 2019. These are part of the new, smarter rules for safer food regulations. They mean that all seed from third countries entering the UK and European Union require documentation. seed is either controlled or prohibited. phytosanitary certificates where required are issued by an officer of the relevant national Plant Protection organisation and on a legal document, indicating that the consignment meets the importing countries legislation standards. a phytosanitary certificate or plant passport does not necessarily mean that seed is pest or pest pathogen free. Where the seed is being managed and stored by a commercial Seed Company, or a seed bank, testing for purity, germability and vigour are standard. The types of testing used to evaluate seed health are much more variable.

Typically, these can include methods such as the cut test, where larger seeds are cut in half, and the presence or absence of invertebrate pests is indicated. An alternative but higher technology method is the use of an X ray machine. testing for the presence or absence of pathogens varies widely. This may be subject to tolerance levels. tolerance levels indicate a level of disease present, which may lead to an acceptable loss in a commercial crop.

At this point, I thought it might be useful to remind ourselves of some of the important seed borne diseases of horticultural crops. These include bacteria, fungi, viruses, and viroids. other diseases are caused by organisms such as nematodes.

External contamination or infestation of seed is often the result of the adherents of particles like fungal spores or sclerotia, which are scleratied bundles of fungal mycelia. sticking to the seed coat, or testa. These can often be managed by surface sterilisation techniques, and are sometimes visible under a hand lens or a microscope.

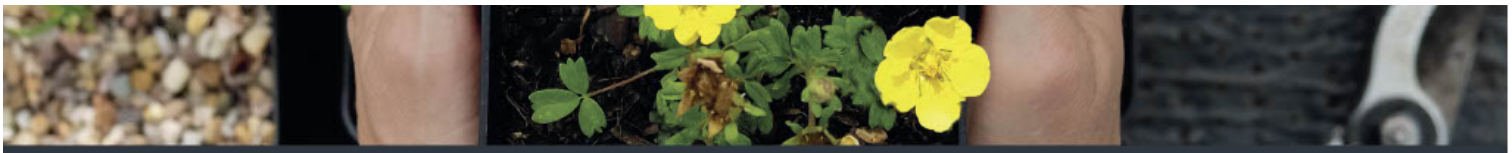
Internal contamination or infection of seed is where the pest or pathogen is carried in the embryo and cotyledons. In the image to the right you can see how a range of apparently normal looking seeds when you subject them to x rays, as below are revealed to contain invertebrate pests. Cut tests, or x rays are useful methods for revealing issues. Incubating seeds briefly can also be a helpful technique, incubating them at elevated temperatures, for a period of days in an airtight container, often elicits weevils, beetles, fly larvae, and others. Commercial seed sorting often relies on gravimetric separation. So seeds that successfully transfer through the process appear to be fine, but in reality, some may still carry pests such as weevils. And you'll see the evidence for this later. work by our colleague in France, has helped to make the case for tighter regulation of the seed trade by demonstrating the clear links between seed and the introduction of non native species to countries. His work here on roses quite clearly shows that wild collected seed, both commercially available and sourced through fieldwork has led to the introduction of a number of alien species to France and to the UK. This work has been supported by more recent studies. Looking at traded forest tree seeds 58 seed lots of angiosperm and gymnosperm seed were tested 30% were found to contain insect larvae; fungi were present in all of the seed plots, and at least 30% of those were potentially pathogenic. More recently, Allen has also looked at the interception data. He determined that this is of limited value, as it focuses on known pests on the A1 and A2 lists. between 1995 and 2012. only seven species of the hundred and 17 established in the European Union, were intersected in trade.

So how can we manage these risks?

When we're collecting seeds, we know that need to follow good hygiene practices. We should collect seed direct from the parent plant ensuring that it looks to be visibly free of pests and pathogens. And we should store seed in a way that avoids the formation of condensation. So paper or fabric bags, we should always avoid wherever possible collecting seeds from the floor. This is where they are likely to accumulate pests, pathogens and saprophytes.

After collection seeds should be handled carefully and cleaned as quickly as possible to ensure that any potential sources of disease inoculum are removed. It should be disposed of carefully. If resources permit test for the presence of pathogens, but you do need to decide whether you will test for the knowns or the unknowns. I would also recommend that you consult a pathologist so that you can understand the implications of any results that you obtain. It's not always obvious.

Prior to seed sowing and germination. There are a number of different options for seed treatment. Ideally, we would always use seed which is confirmed to be disease free, but this is not always possible or practical. Where doubts or concerns exist, then the use of sterilants such as hydrogen peroxide, sodium hypochlorite, which is also known as bleach, or other chemicals can be a useful way of killing any disease inoculum present on the outside of the seeds. methods like hot water treatment, which are typically used for commercial seed crops can be a useful way of killing internal seed pathogens too. But if you get it wrong, you risk killing the pest and the seed too.



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Cultural controls are an invaluable way of tackling biosecurity risks following good hygiene practices. Using high quality growing media, spacing plants, isolating or quarantining seedlots are all useful tools in combating outbreaks of pests or pathogens. constant monitoring, inspection and wroking of plants and identifying any pests or pathogens found are also a key element to this exercise. Managing the growing environment carefully to try and maximise germination success and minimise the likelihood of disease development are also important. It may be necessary or desirable to treat the soil. Separation and treatment of plants can be a useful way of reducing disease inoculum.

Ageing seed prior to sowing is a method that is useful for cucurbits where the seed is known to outlive certain types of pathogen. In short, if you're working with seed, gather information, evaluate the risks carefully. get advice from experts where possible, follow the science. And remember, it's always better, cheaper and safer for everyone. And especially for biodiversity to prevent that The introduction of a pest or disease rather than try to effect a cure. Happy growing, and a very happy 350th anniversary to the Royal Botanic Garden, Edinburgh and many more

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