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Global Impact of International Seed Movement: Regulatory Implications of Seed Health Testing

Synopsis

Molecular tools can dramatically improve seed health testing. However, the presence of pathogen nucleic acids in seed samples does not directly indicate a threat of epidemic development. Hence, concomitant with emerging pathogen detection technology, research is needed to assess the threat posed by seed-borne inoculum. This is important to allow for international seed trade, while reducing the risk of global plant disease dissemination. Unfortunately, with new seed assays, regulatory agencies may set policies based on incomplete information. Hence, it is important to determine how to take advantage of new molecular seed health assays, without losing epidemiological relevance. In response to this concern, we propose a session to explore the implications of molecular detection tools for informing phytosanitary regulation development and enforcement.

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Seed health challenges in the smallholder informal seed system

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Seeds play an important role in the livelihoods of smallholder farmers in many countries, especially in Africa. The freshly harvested seed and/or on-farm stored seed are sold at informal markets for generation of income. Smallholder farmers usually cannot buy good quality certified seed for planting and rely on their own on-farm-saved seed or that obtained from other smallholder farmers for the next season's production. The transfer of poor quality and possibly infected seed is largely uncontrolled. This seed often has reduced germination and seedling vigour, and is vulnerable to fungi, in both storage and the field, which may produce mycotoxins causing serious health complications in both animals and humans. Farmers use different containers (e.g. metal tanks, re-used maize meal sacks) and techniques (e.g. sun drying, seed mixed with ash) to store their seeds. However, fungal infestation of seed due to high temperatures and relative humidity can occur. Effective control and prevention strategies at the level of the smallholder farmer are required, which can be achieved by optimizing storage systems and assistance from trained extension officers. The seed health challenges existing within the smallholder seed system will be reviewed, with emphasis on the challenges faced during postharvest and storage. The negative impact of seed movement across borders due to poor implementation of regulations will also be discussed.

Critical aspects of biologically relevant seed health assays

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There is no question on the importance of preventing the spread of plant pathogens across the globe. This is facilitated by the creation and implementation of phytosanitary regulations, and the continuous improvement of seed health testing protocols. Our experience in developing an assay for detection of *Xanthomonas oryzae* pvs. *oryzae* and *oryzicola* rice seed contamination has shown us that this is not an easy task. Critical aspects of seed health assays are not only limited to the technical specifications of the detection method, such as sensitivity, selectivity, ruggedness, and reliability. In diagnostic PCR, the inclusion of test controls (i.e. internal amplification control, process controls, no template controls, positive controls, and non-target controls) are necessary to validate any result. Also, the simplicity of an assay is important for it to be useful. Is the required equipment commonly available or too specialized? Are the extraction steps simple and easy to carry out? In the final stages before protocol dissemination to users, the protocol should be audited by a higher body, such as the International Seed Testing Association (ISTA), and the accreditation of a protocol for universal use would depend on stringent evaluation by ISTA. Developing protocols for seed health testing should be done with the prime intention to create a method for easy adoption by quarantine laboratories across the globe.

Harmonization of phytosanitary/regulatory policy and seed health testing for safe global seed movement:

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Many pests that are neither seed borne nor seed transmitted are regulated. Individual country seed import requirements differ substantially from one another for the same pest risk. The seed industry is a global business with international operations making it increasingly challenging to meet seed import requirements bilaterally and not multilaterally as befitting international trade. Seeds are a unique group of commodities where most pest risks can be collectively addressed by industry best practices reducing the need for commodity-specific phytosanitary requirements. ISF works to harmonize phytosanitary policy through the development of regulated pest lists, seed health tests, and encouraging recognition of industry best practices as alternative measures, or a systems approach, for import requirements. An extensive review of scientific literature by ISF for seed species with high volume trade shows that seed is a pathway for the introduction, spread and establishment of disease for a fraction of all regulated pests. Seed health testing is a tool used by the seed industry to prevent the introduction and spread of pests known to be associated with seed and ISHI-Veg, an industry-led group, develops internationally recognized seed health tests for seed-as-a-pathway pests. These tests are also part of the quality management practices companies use to protect their seed from exposure to pests and diseases at all stages of seed development, production and commercialization significantly reducing overall pest risk.

Detection of threatening emerging pathogens in maize and wheat seed: Phytosanitary challenges, regulations and solutions

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The Maize chlorotic mottle virus (MCMV) responsible for maize lethal necrosis in East Africa since 2011 and *Magnaporthe oryzae Triticum* (MoT), responsible for wheat blast in the tropical parts of South America since 1985 and in Bangladesh since 2016, have emerged as serious threat to global maize and wheat production, respectively. Seed transmission was suspected to be the mechanism of intercontinental spread of both these pathogens. Reliable testing procedures for pathogen detection in seed lots have become a high priority for enforcing effective phytosanitary controls during seed exchanges. In this study we present development of seed testing methods and regulatory procedures for declaring seed lots and germplasm meant for international exchange free from MCMV and MoT for commercial and research purposes. Enzyme-linked immunosorbent assay (ELISA) for MCMV in maize and a polymerase chain reaction (PCR) with MoT specific primers in wheat were found to be the most reliable and cost-effective methods for pathogen detection in seed lots. Challenges to diagnostic test development due limited knowledge on rate and mode of seed transmission of MCMV, and occurrence of different MoT pathotypes will be presented.

Viruses of *Ullucus tuberosus*: The opportunities and implications of using next generation sequencing in support of statutory diagnostics

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Ulluco (*Ullucus tuberosus*) is a tuber forming crop routinely grown alongside potatoes in the South American Andes, which has become a novel crop in both highland and temperate maritime climates. Eight viruses have been previously reported infecting ulluco, including a strain of Andean potato latent virus (APLV). Ulluco is not currently listed as regulated in EU trade. Following a breach of UK plant health regulations, 400 ulluco plants from a small holding in South-West England were tested for the presence of quarantine viruses using ELISA and real-time PCR. Following positive ELISA results for APLV and multiple other viruses, virus identification was confirmed by Next Generation Sequencing (NGS) using a ribosomal RNA (rRNA) depleted total RNA approach on an Illumina MiSeq platform. Analysis of viral contigs indicated the presence of several novel viruses closely related to, but not consistent with, the viruses indicated by ELISA or those previously described from ulluco. A second outbreak site linked to internet trade in ulluco tubers was investigated using the same approach as described and a similar but distinct suite of viruses was detected. Using NGS in a diagnostic confirmation role offers the ability to identify multiple viruses simultaneously, whilst alleviating the concerns over novel findings without supporting diagnostic context. The limitations of list-based regulatory systems in the era of internet trade will be highlighted.

Cucumber green mottle mosaic virus: Research perspective working with a world travelling virus

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Cucumber green mottle mosaic virus (CGMMV) is a Tobamovirus that infects cucurbit crops and can cause devastating yield losses. CGMMV is primarily spread by contaminated seeds, then transmitted efficiently between plants by mechanical means. This allows geographical movement with the increased international seed trade, then rapid spread into naïve production areas. Originally described in England, global dissemination was slow until 2007, when detections in new regions increased rapidly. In California, the first detection of CGMMV was in 2013, after which annual detections occurred in either field settings or seed shipments. Our research into the genetic variability of CGMMV isolates detected in California indicates significant diversity and new introductions annually. Using Next Generation Sequencing, we have the full genomes of seven California introductions, two of which were identical. Additionally, we sequenced thirty-three isolates originating from Europe and southeast Asia. Incidentally, we identified a new cucurbit-infecting Tobamovirus to California in 2017, which was detected in material presumed to be infected with CGMMV. This virus shares 82% nucleotide identity with Cucumber mottle virus (CuMoV), which was first described in 2006 from Japan. With increasing movement of seeds across international borders, ongoing monitoring is necessary to prevent plant diseases from becoming established in new regions.