Tobamoviruses outbreaks in *Solanaceae* are not linked to use of indicator plant tests: A retrospective analysis.

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Tomato and pepper (fresh chillies and peppers) are grown worldwide and are among the world's most consumed vegetables. FAO reports that global production of tomatoes and peppers in 2020 was 187 million tons and 36 million tons, respectively. Asia has the highest production share of tomatoes by region at 63%, followed by the Americas (13.1%), Europe (12.2%) and Africa (11,9%) (FAOSTAT). Production can be influenced by plant diseases, among other factors.

Major viral diseases in tomato and pepper are caused by tobamoviruses. The *Tobamovirus* genus contains multiple economically important pathogens that infect solanaceous crops. They are considered to be the most stable and infectious viruses known, and are readily transmitted mechanically by workers, tools and equipment during plant handling. Tobamoviruses can also be spread via fruits and insects. They survive in soil, water and infested debris from previous crops. They can be seed borne, however, infection and spread most likely occurs mechanically (Smith and Dombrovsky, 2019).

The spread of the tobamovirus *Tomato brown rugose fruit virus* (ToBRFV) was recently reported by several countries around the world (EPPO Global Database). The virus is causing severe damage to tomato and pepper production sites and immediate actions must be taken by plant raisers and governments to get the spread under control. In 2019, the state of Florida reported that ToBRFV can cause yield losses between 30% and 70% in the state-grown tomatoes, which accounts for $262 million a year in economic impact (WGCU News, 2019). In addition, an experimental study on the effect of the tobamovirus *Pepper mild mottle virus* (PMMoV) on pepper plants showed yield losses between 15% and 40% (Kim *et al.*, 2010).

The total economic impact of plant diseases results from a combination of several factors such as yield losses caused by a reduction in fruit quality and quantity, and the additional costs of crucial hygiene, sanitation measures and unmarketable products.

To produce healthy plants and fruits, producers and plant raisers have access to a toolbox of mitigation measures, such as clean starting material, testing of plants and seeds, resistant varieties and hygiene measures during crop growth.

Seed health testing is frequently used to ensure use and production of healthy seeds. For a seed health test to have predictive value, a risk assessment must take into consideration factors suchas disease dynamics, genetic resistance and epidemiology. The epidemiology of a disease depends upon pathogen infestation levels within and/or on the seed, the climate in which the seed is sown, cultural practices (e.g. direct seeding *versus* transplant production) and the interactions of the host, pathogen and the environment. Measuring the impact of each of these factors on pest transmission is complex as epidemiological studies are often not available and not all growing conditions are known at the time of testing.

Seed health tests and biological relevance
Biological relevance: the presence of living organisms on or in the seed and its pathogenicity. Indicator plant test: infected plant tissue (e.g. seed or leaves) is applied to an 'indicator' plant grown under disease-conducive conditions to assess its infectivity. Grow-out test: seeds are grown under disease-conducive conditions and seedlings are inspected for typical symptoms or tested for presence of the target pathogen.

The most important factor for disease development in a wide range of conditions is the presence of a living, pathogenic target pathogen in/on the seed. Therefore, methods that confirm the presence of a living pathogen in/on the seed and its pathogenicity, such as a grow-out and an indicator plant test (see text box), are good predictors of disease transmission from seed to seedling (Roenhorst *et al*., 2013). Very sensitive methods, such as molecular methods, are frequently used in the seed industry. It should be kept in mind that those tests do not provide information about the presence of living, infectious organisms. A positive test result of a molecular method indicates that the presence of genetic material of a given pest is suspected on the seed, still a follow up test is needed to confirm presence of living and infectious virus particles. Seed health testing based on these principles will help to ensure that healthy seed is available to growers and prevent the introduction and spread of pathogenic organisms (ISF, 2013).

Based on published literature, the seed industry and seed health testing laboratories have developed a robust protocol whereby an indicator plant test based on tobacco plants to detect presence of living and infectious tobamoviruses on tomato and pepper seeds. The indicator plant test is used with or without an ELISA pre-screen on samples of 3,000 seeds (based on ISPM31; IPPC, 2008), with 12 subsamples of 250 seeds (ISF, 2019a, ISF, 2019b; ISTA, 2012). Indicator plants such as *N. tabacum* cv. Xanthi NN are mechanically inoculated with tomato and pepper seed extract, resulting in a hypersensitive response in the form of local lesions on the inoculated leaf surface in case of presence of infectious tobamovirus (ISF, 2019a, ISF, 2019b). In contrast to the more specific molecular methods, different tobamoviruses can be detected with this test, which likely includes upcoming 'new' ones. The indicator plant test for detection of tobamoviruses has been used by seed companies for decades.

To investigate whether the indicator plant test for tobamoviruses is sufficient to prevent the spread of the virus through infected seed, a retrospective analysis of data was done. Several seed companies shared data on the number of indicator plant tests for tobamoviruses in tomato and pepper seed, conducted in the years 2015 to 2019 (see Table 1). An analysis was made of the number of outbreaks related to indicator plant test results. The number of outbreaks was measured as the number of complaints about tobamovirus outbreaks that were received by seed companies. An outbreak that can be traced back to a seed lot that was tested negative in the indicator plant test would be an indication that the indicator plant test is not fit for purpose.

Over 18,000 tomato and pepper seed lots were included in this retrospective analysis. The negative tested seed lots were sold and sown around the globe while the positive tested seed lots were not marketed. A total of 96% of the seed lots tested negative by the indicator plant test. Out of the 17,751 negative seed lots only 2 seed lots, accounting for 0.011%, were investigated due to complaints. Those complaints could not be traced back to infected seed as the cause. This analysis shows that the sensitivity of the indicator plant test is sufficient to prevent outbreaks caused by infected seeds. There were no false-negative results of the indicator plant test. This test was able to distinguish accurately between seed lots that pose a risk in the market and seed lots that do not, based on biological relevance.

When used with laboratory best practices, results reveal that the indicator plant test is a robust tool for predicting risk for *Tobamovirus* infection. Therefore, ISF encourages its continued use to prevent disease outbreaks.

Table 1. Consolidated data (2015 to 2019) of tomato and pepper seed lots marketed based on indicator plant test results.

Seed lots tested for tobamoviruses	Number
Seed lots tested for tobamoviruses using an indicator plant test	18,490
Seed lots that tested negative for tobamoviruses in an indicator plant test	17,751
Seed lots that tested positive for tobamoviruses in an indicator plant test (and were not marketed)	739
Seed lots investigated because of complaints	2
Complaints that could be traced back to infected seed that gave a negative result in the indicator plant test	0

Note: a breakdown of the number of tomato and pepper lots tested is not available but several thousand seed lots of each crop were tested.

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