

Detection and Epidemiology of Pospiviroids

Pospiviroids are plant pathogens, composed only of infectious RNA molecules, which are both latent on many ornamentals and harmful for cultivated plant species like tomato, potato, sweet pepper, citrus or chrysanthemum.

Throughout the world, these pathogens cause sporadic outbreaks on susceptible annual



crops while they often remain undetected although widespread on ornamentals. To assess the risk posed by pospiviroids in EU, the link between this potential ornamental reservoir and the outbreaks on Solanaceae as well as the role of infected seeds in these contaminations have been recently reviewed by the EFSA panel on plant health (PLH) ¹. However, the continuing uncertainty over the role of insects and infected seeds in the spreading of pospiviroids complicated the likelihood assessment of these transmission routes.

On the other hand, the PLH concluded that disinfection measures and the accurate generic detection of pospiviroids are among the indispensable tools for the management of these diseases.

The project DEP2 has focused on these different elements to decrease the uncertainty about the routes of interspecific transmission and seed transmission, evaluate the efficacy of the only approved pospiviroid disinfectant in EU and compare available generic detection methods. The European and Mediterranean Plant Protection Organization (EPPO), is currently working on a Diagnostic Protocol on Pospiviroids and the results from DEP2 will be used to develop the Standard.

Insect transmission

Since the discovery of pospiviroids many studies have focused on the potential role that insects may play in their transmission. In the framework of DEP2, the vectoring capability of the aphid pest *Myzus persicae*, the pollinating agent *Bombus terrestris* and the predatory bug *Macrolophus pygmaeus* was investigated. The results showed that, while individuals of *M. pygmaeus* maintained in contact with pospiviroid infected plants were found negative using RT-qPCR, some tested aphids and bumblebees were positive. Moreover, a transmission event of *Tomato chlorotic dwarf viroid* (TCDVd) from infected petunias to tomato by *B. terrestris* was observed. Even if the isolation of a pathogen from an insect does not guarantee its vectoring capability, nor does experimental transmission necessarily mean that the insect plays an





economically important role in actual disease spread, the experiments carried out in DEP2 project shed some more light on this issue.

Seed transmission

Pospiviroid seed transmission in tomato plants is a controversial subject. Many experiments have been performed and differing results obtained. To investigate the importance of this pathway of infection, DEP2 project has focused on four pospiviroid species: *Tomato apical stunt viroid* (TASVd); *Citrus exocortis viroid* (CEVd); *Columnea latent viroid* (CLVd) and *Potato spindle tuber viroid* (PSTVd). In this part of the project, fruits were harvested from artificially infected tomatoes and more than 7000 seeds were collected. Although the seeds

were found to be infected by the respective inoculated pospiviroids, the about 4.700 seedlings grown from them were all found negative using RT-PCR detection methods. These results, under publication, suggest that seed transmission is not as obvious as previously believed.

Disinfectant efficacy

As pospiviroids are readily transmitted by contact and as affected plants are often cultivated in greenhouses and/or require a lot of handling, efficient disinfection measures to clean the cultivating tools, machineries and facilities have to be found and approved by the plant protection authorities.

Many European authorities approved MENNO® clean, a disinfectant based on benzoic acid for which few data were available. To assess the effectiveness of this disinfectant and compare it with other commercial products, several experiments were carried out in the framework of DEP2.

These experiments showed that MENNO® clean was neither effective at the minimum recommended contact time nor at an intermediate contact time for the minimum recommended concentration. The comparison with the four other commercial disinfectants showed that these products were a possible alternative as effective as a 0.8 % household bleach solution. The experiments conducted in DEP2 also indicated that an appropriate contact between viroid particles and disinfectant active compounds was crucial for disinfection efficacy². Because of the simple chemical nature of viroids, these results could probably be extended to other viroids.

Test performance study of generic detection methods

Because of the absence of Community Reference Laboratories for plant diseases in the European Union, the National Reference Laboratories (NRLs)





themselves have to assess the performance of the detection methods they chose/developed and to compare them with the other existing methods. On the other hand, because most of NRLs try to comply with the ISO 17025 standard, they also have to evaluate their ability to perform these methods on blind samples.

In order to meet these needs, NRLs try to take part in test-performance studies (ring-tests) in which they can test their own methods. However, the lack of available ring-tests, the operational and analytical constraints imposed by the ring-test organizers as well as the paucity of the results and the difficulty to interpret qualitative results often prevent the NRLs to fully assess their methods and clearly identify the strengths and weaknesses of each tested method.

The test performance study organised in the framework of DEP2 was developed and the results were analysed to respond, as much as possible, to the needs of NRLs concerned by the detection of pospiviroids. The results of this comparison, under publication, allowed the participating laboratories to fully assess four different methods with a minimum of constraints while still permitting them to get a detailed overview of performance criteria of each tested method.

References:

- ¹ EFSA PLH Panel (EFSA Panel on Plant Health), 2011. Scientific Opinion on the assessment of the risk of solanaceous pospiviroids for the EU territory and the identification and evaluation of risk management options. EFSA Journal 2011 9, 2330.
- ² Olivier T., Sveikauskas V., Grausgruber-Gröger S, Virscek Marn M., Faggioli F., Luigi M., Pitchugina E., Planchon V. (2015) Efficacy of five disinfectants against *Potato spindle tuber viroid*. Crop Protection 67: 257-260.

Project ID: Assessment of the risk posed by ornamentals and tomato seeds infected by Pospiviroids to tomato crops and evaluation of Pospiviroid detection protocols for seed testing in tomato (DEP2).

