

New insights in freesia leaf necrosis disease

An ophiovirus was found to be associated with freesia leaf necrosis disease. For the first time a virus was successfully isolated from infected freesia and a detection method developed for the isolated virus.

By Martin Verbeek and Ellis Meekes

Freesia leaf necrosis disease (FLN) has been known in freesia cultures for over forty years now. The disease causes severe necrosis of the leaves leading to serious yield losses. Typical symptoms of FLN start as chlorotic spots on freesia leaves, later turning into necrotic spots (Figure 1). Symptom expression however may depend on environmental factors. FLN is soil-borne and is

transmitted by the chytrid fungus *Olpidium brassicae*. The disease can survive in the resting spores of this fungus, which are very persistent and can survive for over 20 years in the soil. No resistant freesia cultivars have been identified so far.

An ophiovirus associated with FLN

For a long time the causal agent of FLN remained

Figure 2. Electron micrograph of purified ophiovirus particles from FLN infected freesia. Bar represents 100nm.

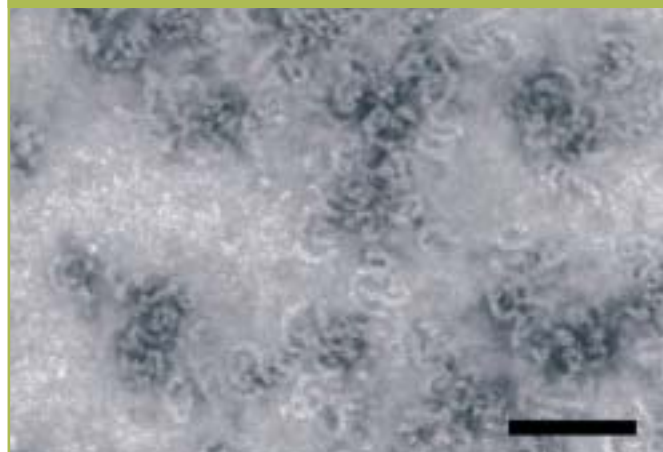


Figure 1. Symptoms of freesia leaf necrosis disease in freesia Blue Moon.



unknown. It was generally assumed that a virus could be involved, but all attempts to prove it failed. Recently we could isolate a virus belonging to the genus *Ophiovirus* from FLN infected freesia plants.

The virus genus *Ophiovirus* is a relatively recently discovered genus. The virus particles (Figure 2) are very difficult to discover by electron microscopy and were for a long time not recognized as such. Actually, the virus particles consist of RNA, wrapped in protein. The particles are long and small treads and have a 'snaky' appearance. This is why this genus is called *Ophiovirus* (ophis means snake in Greek). In the recent past ophioviruses are found in e.g. citrus (*Citrus psorosis virus*, CPsV), tulip (*Tulip mild mottle mosaic virus*, TMMMV) and lettuce (*Mirafiori lettuce big-vein virus*, MLBVV, and *Lettuce ring necrosis virus*,

LRNV). Now, we have also found an ophiovirus in freesia with leaf necrosis disease.

Virus isolation and development of a detection method

One of the characteristics of ophioviruses is that the particles are extremely unstable and therefore difficult to purify and handle. Standard mechanical inoculation methods to transfer the virus to indicator plants were not useful. New inoculation methods were developed to transmit this ophiovirus and several indicator plants were tested as alternative host plants. Two new alternative hosts were identified: *Nicotiana glauca* '67A' and *Nicotiana glauca* 'P1'. Initially these hosts only show local lesions on the inoculated leaves, but after multiple transfers the virus was capable to infect the tobacco plants systemically

(Figure 3). After systemic infection the alternative hosts showed high virus titres and proved suitable for use in also newly developed virus purification procedures. The purified virus was used for raising a polyclonal antiserum. This antiserum reacts very well with the virus when used in standard ELISA tests, and can distinguish clearly between healthy and infected freesia samples.

Evaluation of the detection method

Now a detection method is available, Naktuinbouw is evaluating the antiserum and conducting surveys. By testing many field samples, the relations between ophiovirus infection and necrosis symptoms can be investigated. Direct evi-

dence that this ophiovirus is the causal agent of FLN is not yet available, because mechanical inoculation of the isolated ophiovirus back to healthy freesia was not successful so far. However, the first test results on freesia field material are very promising and indicate that the found ophiovirus is at least associated with FLN. The developed detection method will be an essential tool for breeders in their resistance breeding programs. ■

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Figure 3. Systemic symptoms of freesia ophiovirus in *Nicotiana occidentalis* P1.

