Black death in hellebores: Characterization of a virus disease in *Helleborus* x *hybridus*

Dr Béatrice Henricot, Plant Pathologist

Hellebore black death has been known for at least 10 years by hellebore growers but its cause has been difficult to prove. The symptoms are black spots or streaks on the leaves, stems and flowers. The plants become stunted and may die. This disease is mainly found on various cultivars and hybrids known to gardeners as *Helleborus orientalis* now called *Helleborus* x *hybridus*, but too little is known about the situation to comment on the susceptibility of other hellebore species. The aim of this project is to determine the exact cause of this disease and to examine the host range, the means of spread, infection and control of the disease.

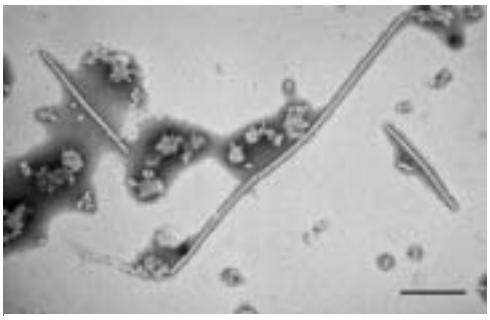
Symptoms of hellebore black death

Research summary

For the first time, since the problem of hellebore 'black death' was reported at the beginning of the 1990s, virus particles were observed in plants showing typical symptoms. The most abundant particles in the infected tissues are flexuous rods. Antibodies produced against the virus can now be used in enzyme-linked immunosorbent assay (ELISA) to detect the disease from hellebore stocks. Two common plant viruses, tomato mosaic virus (ToMV) and cucumber mosaic virus (CMV), not recorded before in hellebores, have been detected in some infected plants. The involvement of these viruses in the disease is so far unknown**.** Further work aims to clone part of the genome of this virus to enable its identification.

How did we detect virus particles?

Filamentous rod-shaped virus particles were observed from diseased *Helleborus* x *hybridus* using transmission electron microscopy.



Virus particles from *Helleborus* x *hybridus* examined under electron microscopy (bar=300 nm)

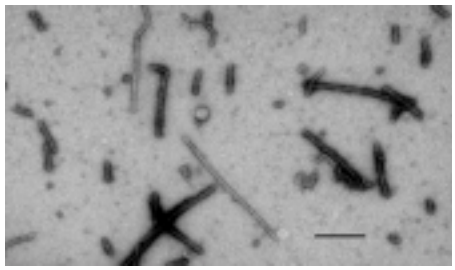
Is this virus related to other known viruses?
Sap of infected hellebores was tested for other known viruses using the technique ELISA (enzyme-linked immunosorbent assay). Known viruses tested were:

AlMV (*Alstroemeria mosaic virus*), AMV (*Alfalfa mosaic virus*), CMV (*Cucumber mosaic virus*), CNFV (*Carnation necrotic fleck virus*), CRSV (*Carnation ringspot virus*), CVB (*Chrysanthemum virus B*), LSLV (*Lily symptomless virus*),	potyvirus (general), TAV (*Tomato aspermy virus*), ToMV (*Tomato mosaic virus*), TMV (*Tobacco mosaic virus*), TuMV (*Turnip mosaic virus*) TSWV (*Tomato spotted wilt virus*).

The only positive reaction was seen with antibodies to CMV and ToMV but only in a small percentage of the tested plants. Particle types typical of these viruses have been observed in the infected material among the filamentous rod-shaped particles.

Molecular methods were also used to investigate relationships to other known viruses. Reverse transcriptase polymerase chain reaction experiments (RT-PCR) using degenerate primers (used for the diagnosis of typical filamentous rod-shaped viruses such as poty-, clostero- and carlaviruses) failed to amplify RNA typical for these virus groups. However, some nucleic acid amplification occurred with carlavirus specific primers which suggests that this virus is closely related to, or belongs to, this group.

How do we test for the disease?
Filamentous rod-shaped particles were purified from infected tissues and used to produce antibodies. The antibodies were tested successfully using the technique ELISA. The antibodies detected the virus particles in infected plants but not in symptomless plants.



Flexuous rods from an extract of naturally infected *Helleborus* x *hybridus* are 'decorated' (bar=300 nm).

To visualise that the antibodies recognised the flexuous rod-shaped particles found in the infected material, the technique of decoration electron microscopy was used (Fig.3). The electron microscope grid was first coated with antibodies produced against the hellebore virus. This bound the viral particles, which were then treated with further antibodies. The effect of a halo of antibody molecules around the virus particles, or 'decoration', confirmed their specific detection.

How is it transmitted?
Artificial inoculations using mechanical transmission or the hellebore aphid (*Macrosiphum hellebori*) are under way to confirm that these virus particles are the cause of the damage. Mechanical transmission is carried out by applying an abrasive material to the leaf surface and then the infected sap. It appears that the virus is not mechanically transmissible to hellebore or other indicator plants. Hellebore aphid is a strong candidate as the vector of the disease during sap feeding activities.

Gardeners' advice
There is no treatment currently known for this disease. Infected plants should be removed and disposed of.