Sweet chestnuts can suffer heavy losses due to the parasitic fungus *Cryphonectria parasitica*.

Cryphonectria parasitica

During the IDS tour of northern Greece in April 2015 members visited the old chesnut forest on Mt Menoikion. The following article is based on the notes taken by **GRISELDA and WILLIAM KERR** during the discussion that took place, when Dr Stephanos Diamandis showed the group the symptoms of chestnut blight. He explained the disease and told them about the success of biological control. These notes were kindly revised by **DR DIAMANDIS**.

This alien parasite, which originated in China, was responsible for killing almost 100% of the American chestnut (*Castanea dentata*) in the USA in the first half of the twentieth century (1.3 billion trees). In 1938 it entered Europe through Italy with similar potential long term effects. Since 1963 when it was first detected on Mt Pelion, the disease has caused severe damage to the sweet chestnut (*Castanea sativa*) forests and orchards all over Greece (see above). However, around 1955 and quite surprisingly, it was found that some trees in Italy with cankers were not killed. Research showed that a weakened, white form of the fungus created superficial cankers without affecting the health of the trees. In the following years it was found that this white form was naturally infected by viruses of the genus *Hypovirus* with dsRNA which converted the orange-yellow, virulent strain of *Cryphonectria parasitica* into hypovirulent


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and rendered it non-lethal to the trees. In the last 40 years such natural hypovirulent forms were found in other European countries too.

This unique case of viral infection of a parasitic virulent fungus and its conversion to hypovirulent (non-lethal to the tree) is now used as a means of biological control. The fungus *Cryphonectria parasitica* shows in nature wide diversity expressed as vegetative compatibility types (vc types). The fewer vc types of the fungus that occur in a region or country the more successful the biological control may be. Inoculations were applied first in France in the late 1980s and later in Italy. Dr Diamandis and his team started surveying the entire country in 1995 and found only four vc types, those of EU-1, EU-2, EU-10 and EU-12, as opposed to the USA where the official number of vc types is over 70. The conclusion was reached that application of biological control on a wide scale was possible in Greece. Luckily, in 1986 a few trees with the hypovirulent form were found in Mt Pelion (Central Greece) at the original entrance place of the parasite. This indigenous hypovirulent strain was isolated and used in the application of biological control.

Biological control consists of introduction of the hypovirulent form of the fungus into chestnut areas where it has not appeared naturally. The procedure is to:

1. Isolate the local virulent strain/s from an area (e.g. prefecture) and identify the vc types they belong to,
2. introduce the virus and convert it/them into hypovirulent *in vitro*,
3. grow the hypovirulent form in a large quantity and make it into a thick paste,
4. Return to the area and inoculate accessible cankers around their perimeter creating a defence line (see photo above).

The entire technique was refined resulting in almost 100% successful establishment of hypovirulence. The paste is administered by injection into 5 mm holes surrounding the canker. It is important to make sure that the canker is completely encircled by injected holes (see photo above). A special instrument for this purpose has been developed by Dr Diamandis to make it swift and simple for the contractor who administers the paste.

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Opposite, the effect of inoculation with a hypovirulent strain of the fungus is to stop the canker growing at the defence line and *right*, healing tissue is being produced around a large canker.

When the canker grows and reaches the defence line, the virulent form of the canker and the hypovirulent form of the defence line anastomose, and since both forms are compatible (they belong to the same vc type) the virus with dsRNA passes into the virulent form. The virulent form is converted to hypovirulent, the canker stops growing and the tree starts producing healing tissue and gradually heals the canker (see photo above).

In areas where more than one vc type is found, the paste is a blend of compatible hypovirulent strains which means that regardless of the vc type on each canker the paste will still be effective. There are certain restrictions in the criteria used when inoculating diseased trees. Trees of between five and 15 years old with smooth bark are selected, as with trees of that age it is possible to clearly define the boundaries of the canker so that the paste can be inoculated around it at a distance of 3 cm outside the canker edge. Following treatment the canker begins to reproduce spores of the hypovirulent form and these are carried to other affected chestnut trees in the area. This is significant because it means that not every tree needs to be treated. The density of inoculated trees in Greece is 70 to 90 trees per ha. The treatment is repeated in the same stands and orchards for three consecutive years. Taking into account the heavy loss, 17 prefectures were inoculated in the period 2007–2009. The results evaluated in 2011 were successful beyond any expectation. Eleven more prefectures are currently being treated (2014–2016).

Dr Diamandis has a team of four scientists who produce the variety of paste that is required. In the frame of a collateral project with Romania, he extended his technique to the region of Maramures with great success. This seems to be an extremely edifying story demonstrating swift, efficient and relatively low-cost treatment of a potentially economically devastating disease.