Methods Used for Investigating the Incidence of *Phytophthora* Disease of Alder in Hungary

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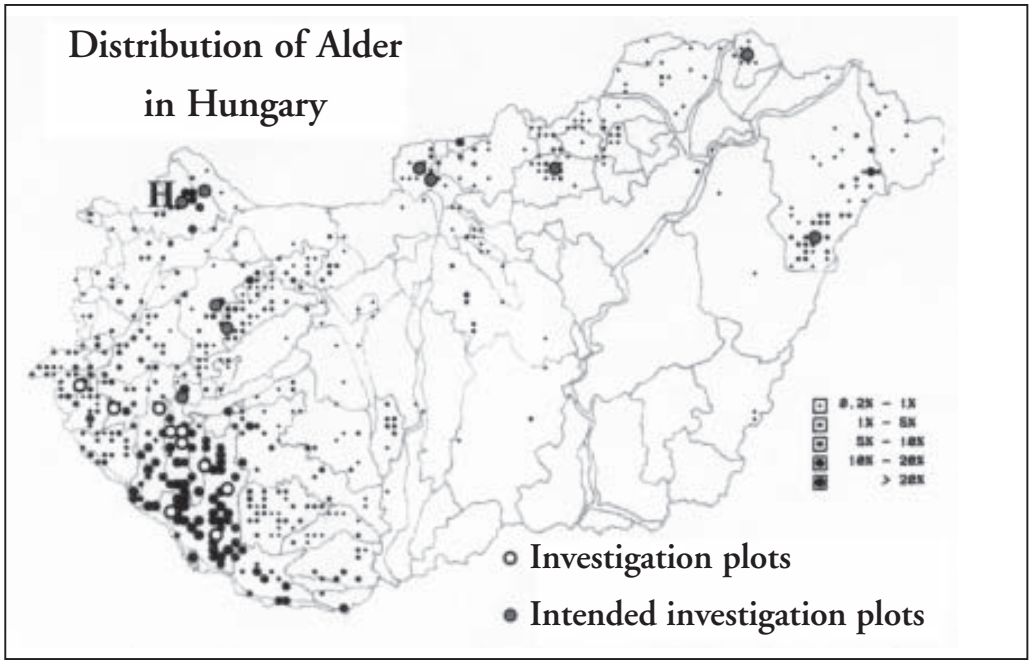

Phytophthora decline of alder was first observed in Hungary in 1999 in Hanság (NW Hungary, close to the Austrian border –designated by "H" on the map.

The Department of Forest Protection, Hungarian Forest Research Institute (FRI) started a long term monitoring project in 2001 on *Phytophthora* decline of alder, specifically to determine the importance of the disease, the extent of damage, and severity of the infection in different regions of the country.

During preliminary investigations in 2001, 57 fen type alder forests and riparian type alder forests were investigated over a distance of 100 km. Results of this investigation were reported by Koltay (2002) in Prague. Experimental plots are being established in 2002 in order to determine presence/absence of the disease, and to measure the rate and intensity of the infection. Ten new plots have been established to date and another 10 plots will be established in the future (*see map*). Stands that contained more than 3% of infected trees in different age classes and of different origins were selected as experimental plots. All plots contain 50 sample trees with the exception of two plots which contain 100 trees. Up to now we have examined each site once per year, but beginning in 2003 we will visit the plots twice each year (late spring and autumn).

Investigative methods are as follows:

Data sheet for each tree

- Location of plot
- Number of plot
- Date of investigation
- GPS code
- Tree number
- Age
- Origin - *Seed, Coppice, or Mixed*
- Diameter at 1,3 m
- Height class by Kraft (1-4)

- ALNUS (1-5) - General condition of the tree on the strength of crown condition
  1. *Previously killed tree, usually without dead twigs*
  2. *Recently killed tree, without leaves but with some dead twigs.*
  3. *Typical decline symptoms in the crown, reduced foliage with leaves smaller than normal, abundance of dead twigs and branches*
  4. *Initial symptoms of crown decline, sparse crown, and in many cases leaves smaller than normal.*
  5. *Healthy and full crown, with green, normal sized leaves*

- Leaf cast (0-10), *measure of lost leaves, 0 = no leaf cast, 10 = 100% leaf cast*
- Leaf chewing (0-1), *0 = No, 1 = Yes*
- Leaf discoloration (0-1), *0 = No, 1 = Yes*
- Leaf measurement (0-1), *0 = No, 1 = leaves smaller than usual*

- Twig dying (0-1), *0 = No, 1 = Yes*
- Branch dying (0-1), *0 = No, 1 = Yes*
- Top drying (0-1), *0 = No, 1 = Yes*
- Abnormal fruiting (0-1), *0 = No, 1 = Yes*

- Tarry spots (0-1-2), *0 = No, 1 = Yes, 2 = Not clear*
- Number of tarry spots on root swelling, *from the soil to 50 cm high on trunk*
- Number of tarry spots on trunk, *higher than 50 cm*

- Wound on stem, (0-1), *0 = No, 1 = Yes*
- Wound callus on stem, (0-1), *0 = No, 1 = Yes*
- Insect damage on stem (0-1), *0 = No, 1 = Yes*
- Water sprout (0-1), *0 = No, 1 = Yes*

In addition to monitoring the incidence of *Phytophthora* decline of alder in Hungary, we have begun identifying *Phytophthora* from our experimental plots in different regions of Hungary. These investigations are conducted in laboratories of the Plant Protection Institute, Hungarian Academy of Sciences.

Isolation methods are described below:

Soil samples containing infected roots were collected around obviously declining trees that exhibited typical tarry exudation on the lower stem. Two or three subsamples were taken from different cardinal directions at 0.5-1.5 m from the stem and were mixed. Isolates were baited by leaves of common Cherry-laurel (*Prunus laurocerasus*) according to Themann and Werres (1998). Symptomatic leaves were then washed under running tap water, disinfected in 10% sodium hypochlorite, rinsed in sterile tap water and dried on paper towels. Pieces of leaves were then placed on selective pea-broth agar (Tuite 1969) amended with 250 mg ampicillin, 10 mg pimaricin, 10 mg rifampicin 50 mg benomyl, 50 to 100 mg PCNB and 50 mg hymexazol per litre.

In addition to baiting, isolations were also made by direct plating of washed and surface disinfected root pieces onto selective media. Plates were incubated at 25 °C in the dark. Developing colonies were purified from contaminants by repeated transfer of the cultures onto the selective medium or 3% water agar (Abdelzaher et al. 1994). Pure cultures were subjected to morphological examinations.

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