# Ink disease in chestnuts: impact on the European chestnut

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#### Abstract [Review article]

A resurgence of ink disease in chestnuts in Europe has been noted since the end of the last century. This lethal disease is currently limiting the establishment of new groves and is threatening the survival of old ones. This paper discusses the impact of the disease and current knowledge about its aetiology and epidemiology. It gives information on the existing variability in tolerance in selected hybrid clones and in wild populations.

Keywords: *Phytophthora* spp., *P. cinnamoni, P. cambivora,* resistance, ink disease, chestnut, *Castanea sativa* Mill.

### 1 Introduction

Ink disease is one of the most destructive diseases affecting *Castanea sativa* Mill. It causes root and collar rot of adult trees and of seedlings in nurseries, plantations and forests. Symptoms of the disease on adult trees include chlorotic leaves reduced in size, thinning of the crown, and immature husks remaining on the tree after leaf-fall. Flame shaped dark necroses are evident on the collar of the tree after debarking. It is the large roots that are mainly infected. They produce a black exudate that stains the surrounding soil, especially during spring and fall. On young trees with smooth bark, the necroses are visible without debarking as depressed, slightly cracked areas at the base of the stem. Infected seedlings in nurseries or plantations undergo a rapid or gradual wilting of the leaves. In the root system, there is extensive necrosis of the tap root that extends to the lateral roots and up the stem for some centimetres.

The first record of the disease was in Portugal in 1838, although it is believed to have been present in Spain since 1726 (CRANDALL 1950). It has since been reported in many European countries, including Spain, Italy, France and the United Kingdom. Two species of *Phytophthora* have been found to be responsible for ink disease in Europe, namely *P. cambivora* (Petri) Buis and *P. cinnamomi* Rand (PETRI 1917, MILBURN and GRAVATT 1932, DAY 1938, CRANDALL *et al.* 1945). The genus *Phytophthora* was formerly attributed to the kingdom of fungi (PETRI 1917), but today it is considered to belong to the Chromista kingdom in the Phylum Oomycota (HAWKSWORTH *et al.* 1995).

Before the epidemic spread of the chestnut blight fungus (*Cryphonectria parasitica*) in Europe in the '30s, ink disease was considered the primary threat to chestnut survival in Europe. In 1923, the Italian Government issued a law (D.M. 01/07/23) that imposed measures to eradicate and prevent the disease. Different methods were developed by growers to control ink disease such as the "Gandolfo" method (named after a chestnut grower from Northern Italy) that involved exposing large infected roots to the winter frost in order to kill the pathogen. During the chestnut blight epidemic, little attention was paid to ink disease, and few experimental and scientific papers have been published on this topic in the last 50 years.

However, in the last five to six years high mortality of *C. sativa* trees with symptoms of ink disease has been reported from different areas in Europe (ABREU 1996, ANSELMI *et al.* 1996). In some cases the disease has limited the establishment of new groves or the conservation of the old ones. A renewed scientific interest in this old disease has been stimulated by its resurgence, and attempts are being made to find out more about its actual distribution, epidemiology and aetiology.

The COST action G4 "Multidisciplinary chestnut research" has contributed greatly to the collaboration of scientists researching ink disease and to establishing national and international research projects. The following discussion describes the current status of ink disease in Europe since it first reoccurred at the end of the last century.

#### 2 Distribution of ink disease in Europe

Recent information from a large number of European countries (COST G4) has contributed to an updated distribution map of ink disease in Europe. Ink disease has been recorded in Italy (VETTRAINO *et al.* 2001), France (DÙFRÉNOY 1924, MOREL *et al.* 2001), Spain, Portugal (CRANDALL 1950), Greece, Switzerland (ARRIGONI 1950, REICHARD and BOLAY 1986), the United Kingdom (DAY 1938, FOISTER 1940), Turkey (BIÇICI and CINAR 1999), Hungary, Slovakia (JUHÁSOVÁ 1999), Romania (BOLEA *et al.* 1995), Macedonian, and Azerbajan (Fig. 1). It is apparently absent in Bosnia-Herzegovina, Croatia and Germany (Table 1). According to the Global Module ( 2<sup>nd</sup> Edition © CAB International Wallingford, UK, 2000), the occurrence of ink disease overlaps with the distribution maps of *P. cambivora* and *P. cinnamomi*, the two main species considered responsible for this disease. No information on ink disease distribution is currently available for other European countries.



Fig. 1. Distribution map of ink disease on *Castanea sativa* based on the information provided by COST G4 participants. Countries where the disease has been recorded are marked in grey.

### **3** Species identification and pathogenicity

*Phytophthora cambivora* and *P. cinnamomi* are the two species most commonly associated with the disease in Europe (Table 1) and considered the most pathogenic to *C. sativa*. These species are able to kill adult trees in one to three years. *P. cactorum* and *P. citricola* have been recently isolated from soils in chestnut stands affected by ink disease. (BIOCCA *et al.* 1993, VETTRAINO *et al.* 2001) Pathogenicity tests through soil infestation on one-year-old *C. sativa* seedlings ranked *P. cinnamomi* and *P. cambivora* as the most aggressive species, followed by *P. citricola* and *P. cactorum* (Vannini data unpublished, VETTRAINO *et al.* 2001). Other *Phytophthora* species have been occasionally isolated from soil and baits and include *P. gonapodyides* (VETTRAINO *et al.* 2001), *P. syringae* and *P. cryptogea* (Vannini, data unpublished). However their role in ink disease is still unclear.

The relative occurrence of the two most pathogenic species varies according to country. In France, *P. cinnamomi* is the most prevalent species in orchards, natural stands and nurseries, although *P. cambivora* has also been frequently isolated (MOREL *et al.* 2001). In Portugal, *P. cinnamomi* has also been the species most frequently isolated, wheras *P. cambivora* has only occasionally been found (MARTINS *et al.* 1999). In Italy, *P. cambivora* is the most prevalent species in orchards and natural stands (VETTRAINO *et al.* 2001, VANNINI and VETTRAINO 2001). *P. cinnamomi* was responsible for tree mortality in a coppice in the Southern Latium region (CRISTINZIO 1986) but no other records of this species in natural stands and orchards are currently available. *P. cinnamomi* is, however, present in nurseries in several areas of Italy (TURCHETTI and PARRINI 1993, PARRINI *et al.* 1997).

Country	Ink disease				Main <i>Phytophthora</i> species associated with ink disease
	Date of first				
	record	Present	Absent	Unknown	
Italy	1917	+			P. cambivora, P. citricola,
					P. cactorum, P. cinnamomi
France	1924	+			P. cinnamomi, P. cambivora,
					P. citricola, P. cactorum
Spain	1726	+			P. cinnamomi, P. cambivora
Portugal	1838	+			P. cinnamomi, P. cambivora,
					P. cactorum
Greece	unknown	+			P. cambivora
Switzerland	1940	+			P. cambivora, P. cinnamomi
United Kingdom	1938	+			P. cambivora, P. cinnamomi
Turkey	1925	+			P. cambivora
Hungary	unknown	+			No information
Slovakia	1974	+			P. cambivora, P. cinnamomi
Romania	1985	+			P. cambivora , P. cinnamomi
Macedonian	unknown	+			No information
Azerbaijan	unknown	+			No information
Bosnia-Herzegovina			+		No information
Croatia			+		No information
Germany			+		No information
Austria				+	No information

Table 1. Records of ink disease on *Castanea sativa* and the most frequently associated *Phytophthora* species in Europe based on the information supplied by COST G4 participants.

#### 4 Climatic and human factors predisposing trees to ink disease infection

Several studies have been carried out in Portugal on the effect of climatic factors and human practices on the incidence of ink disease caused by *P. cinnamomi* in *C. sativa* groves. These studies showed that incidence of the disease was higher in southerly exposed sites and in poorly aerated and depleted soils (MARTINS *et al.* 1999). A negative correlation was found between disease incidence and lime and manure fertilisation of the soil (PORTELA *et al.* 1999).

In Italy, the spread of ink disease in coppices and orchards is associated with the network of rural and public roads that transverse the chestnut forests (ANSELMI *et al.* 1999). Furthermore, as VETTRAINO *et al.* (2001) showed, it is only possible to recover *P. cambivora* easily from soil in April-May and September-October. It is at these times that there is the highest risk of the disease spreading.

## 5 Resistance of *Castanea* spp. to ink disease

Asiatic chestnuts (*C. crenata* and *C. mollissima*) were introduced into France in 1925. At this time quarantine regulations were set up in order to prevent *Cryphonectria parasitica* entering the country, and introductions of plants were forbidden. Several trials showed the high tolerance of these exotic species to ink disease and their poor adaptation to French edaphic and climatic conditions (SCHAD *et al.* 1952). In 1952, a breeding programme was started. Its main objective was to create and select interspecific hybrids obtained from open or controlled crossing to be used as resistant rootstocks.

Clones with a high a tolerance to *P. cinnamomi* were selected as rootstocks for high quality fruit cultivars. The most common of these are "Marsol" (CA07), "Maraval" (CA74), "Ferosacre" (CA90), "Marigoule" (CA15) and "Marlhac" (CA118) (SALESSES *et al.* 1993). These hybrids are *C. crenata* x *C. sativa* or the reciprocal. These resistant clones are, however, genetically incompatible with clones of *Castanea sativa*, which has limited this utilisation outside France (CRADDOCK and BASSI 1999). In the last 15 years, more hybridisation programs have been initiated in France, Spain and Portugal in order to select hybrids that are not only tolerant to *Phytophthora* but also vigourous and producing high quality nuts (GUEDES-LAFARGUE and SALESSES 1999, GOMES-PEREIRA *et al.* 1993). A diallele cross between *C. crenata* and *C. mollissima* has been carried out in France to study the mechanisms of tolerance to ink disease (GUEDES-LAFARGUE and SALESSES 1999).

A large screening of the half-sib progenies of *C. sativa* from different European countries, including France, Italy, Spain, Greece and the United Kingdom, is currently being undertaken in order to assess the variability in tolerance of ink disease of the wild chestnut population in Europe (CASCADE EVK2-CT-1999-00006). Preliminary data from this screening program indicates the presence of relatively high variability in response to the pathogen challenge amongst the chestnut population tested to date (Vannini data unpublished).

#### 6 Concluding statement

Research on ink disease carried out in recent years has shown that a variety of factors influence the progression of the disease in natural stands and plantations. Variables such as the *Phytophthora* species involved, the climatic conditions, the human impact and the genetic structure of the host population could account for the severity of disease outbreak in a certain areas. There is an urgent need to develop reliable methods to predict and control the progression of ink disease. Thus more effort should be devoted to research activities focusing in on epidemiology, host predisposition to disease, and pathogen activity and survival.

#### Acknowledgements

This study was carried out with financial support from the project EVK2-CT1999-00006 "Securing gene conservation, adaptive, breeding potential and utilisation of a model multipurpose tree species (*Castanea sativa* Mill.) in a dynamic environment". We are very grateful to COST action G4 "Multidisciplinary Chestnut Research" for providing us with the opportunity to meet other scientists involved in ink disease research and to exchange ideas and information.

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Accepted 15.11.01