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BLIGHT AND INK DISEASE AS CONSTRAINT FACTORS IN CHESTNUT STANDS OF MEDITERRANEAN AND CENTRAL EUROPE

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Abstract

Chestnut stands located in France, Italy, Hungary, Slovenia and Slovakia were surveyed for their phytosanitary situation. Chestnut blight is the most common disease in these woods. In the past, *Cryphonectria parasitica* has caused severe damage both in France and Italy but spontaneous re-growth of the blighted trees is occurring in almost all the visited stands. In Slovenia, Hungary and Slovakia the disease has been spreading recently. Healing and healed cankers and hypovirulent strains of the parasite are present in many of the investigated chestnut woods and a reduction of damage was related to their spread in all the investigated countries. Several intrusive species of trees are in competition with chestnut trees in abandoned stands. Ink disease, caused mainly by *Phytophthora cambivora*, was observed both in France and Italy stands and its damage appears very effective and increasing. The effects of both the diseases on the evolution and development of chestnut woods are evaluated and their sustainable management is discussed.

Keywords: Chestnut forest, *Cryphonectria parasitica*, *Phytophthora* spp., disease, forest health status, hypovirulence, natural evolution

KOSTANJEV RAK IN ČRNILOVKA PRAVEGA KOSTANJA KOT OMEJUJOČA DEJAVNIKA V KOSTANJEVIH SESTOJIH MEDITERANA IN SREDNJE EVROPE

Izvleček

Opravili smo popise zdravstvenega stanja kostanjevih sestojev v Franciji, Italiji, Sloveniji, na Madžarskem in Slovaškem. Kostanjev rak je najpogostejša bolezen v teh sestojih. V preteklosti je Cryphonectria parasitica povzročala močne poškodbe v kostanjevih sestojih v Franciji in Italiji. Vendar je v večini pregledanih sestojev prišlo do ponovnega odganjanja dreves, ki jih je v preteklosti okužil kostanjev rak. V Sloveniji, na Madžarskem in Slovaškem se je bolezen razširila šele pred kratkim. Na mnogih izmed pregledanih ploskev so bila prisotna ozdravljena rakasta drevesa in hipovirulentni sevi parazita, čemur smo pripisali zmanjšan obseg poškodb. V opuščenih sestojih se pojavlja večje število drevesnih vrst, ki konkurirajo kostanju. Črnilovko pravega kostanja, ki jo večinoma povzroča Phythophthora cambivora, smo opazili v sestojih v Franciji in Italiji; poškodbe, ki jih povzroča, so zelo učinkovite, njihova pogostnost pa narašča. V prispevku obravnavamo vpliv obeh bolezni na razvoj kostanjevih sestojev in na trajnostno gospodarjenje z njimi.

Ključne besede: kostanjev gozd, Cryphonectria parasitica, Phytophthora spp., bolezen drevja, zdravstveno stanje gozda, hipovirulenca, naravni razvoj

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VSEBINA

1 INTRODUCTION
UVOD

European chestnut (*Castanea sativa* Mill.) is an economic resource for many mountainous areas of Southern and Central Europe. Products such as lumber, furniture, poles, fences, railroad ties and tannins for leather goods are the most important commercial products from the species. Nuts are produced for human food and animal feed. European chestnut also is a highly prized landscape tree.

Socio-economic changes and the spread of severe diseases have caused the decline in chestnut cultivation of many European regions. After a long period of abandonment, the interest and the economic importance of nut production is increasing and the renewal of chestnut cultivation is growing. The spontaneous re-growth of chestnut trees is occurring in most chestnut stands, which are in a semi-cultivated or abandoned condition, and is favoured by the decline in virulence of chestnut blight (BONIFACIO / TURCHETTI 1973).

Cryphonectria parasitica (Murr.) Barr, the blight causal agent, is the main constraint factor of European chestnut ecosystems, but the evolution of this disease is unusual. After the first severe stage of the epidemic, the natural spread of hypovirulence has allowed many chestnut stands to recover spontaneously (HEINIGER / RIGLING 1994, TURCHETTI / MARESI 1990). This new situation has increased interest for chestnut cultivation in Europe.

Regarding this, investigations are starting to collect knowledge in the field to verify the effective role of the diseases on the evolution of chestnut stands and on their sustainable management, focusing particularly on: the presence and effects of the blight and of ink disease dissemination in chestnut stands of Italy, France, Slovenia, Hungary and Slovakia; hypovirulence spread in the selected plots and characterisation of the *C. parasitica* strains.

2 MATERIAL AND METHODS
MATERIAL IN METODE

In the field

Visited areas Chestnut orchards and coppices were visited in France, Italy, Slovenia, Hungary (Zalaegerszeg County), Slovakia and detailed investigations were carried out in stands placed in Cévennes (France) and in Tuscany (Italy). Chestnut stands were observed during the period from 1999 to 2001. The altitude of the selected sites was between 350 and 850 m above the sea level. The majority of the examined plots were growing in the natural *C. sativa* range while Hungarian and Slovakian chestnut stands could be considered as its northern border.

Different chestnut stands and orchards were visited in Slovenia, Hungary and Slovakia, while in France and in Italy the investigations were carried out in chestnut sites selected in the Cévennes Area (France), in Mugello, Monte Amiata and some observations were done in Garfagnana (Italy) and in Ardèche (France). Vegetative conditions and total or partial (as carrying some cures to make easier the chestnut fruit harvest) abandonment were noted. The presence of intrusive and competitive tree species was recorded in the selected sites.

Chestnut coppices were visited in the Cévennes Park (France) and a permanent chestnut coppice plot was observed at Pallières.

In Italy ten chestnut coppices placed on the typical chestnut areas of Tuscany (Mugello, Monte Amiata and Garfagnana) were visited. On Monte Amiata the phytosanitary aspects of an experimental conversion from a coppice to a chestnut forest, by cutting the dominated or dead sprouts, were recorded.

A coppice located near the same area is converting into a chestnut orchard by grafting, for experimental purposes, some Italian 'marrons' varieties on selected sprouts of each chestnut stump. The union point is protected by a biological wax. In Hungary, the same experiment is being carried out in a coppice located near Botfölde (Zalaegerszeg County) to verify the effectiveness of the biological wax against *C. parasitica* attacks on the grafts.

The damage caused by the blight and ink disease was observed and evaluated as the mortality levels caused by these diseases.

The different blight infections (normal (N) or virulent (V), abnormal (A) and intermediate (I)) were detected and their incidence determined.

Samples of the different cankers were collected in each of the examined chestnut plots for the laboratory investigations.

Ink diseased bark samples were collected in infected sites for isolation of *Phytophthora* spp.

In laboratory

Different cultures of *C. parasitica* were obtained from the samples collected in the examined Regions and Countries. Morphological characters of these cultures were observed on PDA (Difco) medium with biotin (1 mg/l) and methionin (100 mg/l) added (PDA mb).

Virulent, hypovirulent and intermediate cultures were observed for morphological characters and pycnidia production on PDAmb. The hypovirulent strains, unable to sporulate, were assayed on segments of chestnut stems placed in Petri dishes containing PDAmb. After one month, these cultures were observed for the presence of pycnidia.

To test for *phenol oxidase activity* the strains were grown on Bavendamm's medium. It contains 0,5% tannic acid, 1,5% Difco Malt extract, 2% Difco Bacto agar, as indicated by RIGLING / HEINIGER / HOHL (1989).

In the *vegetative compatibility (V-C) tests*, the virulent (V) or normal (N) strains were paired with some previously selected Italian testers. Tests were carried out placing the pairs on the medium surface and incubated at 27°C, in the dark, for 15 days, as indicated by ANAGNOSTAKIS (1977). Repulsion between the cultures or a pycnidia production on the colony margin (barrage) was evaluated as a result of incompatibility.

Virulent (V) and hypovirulent (H) strains were grown on cellophane sheets placed on PDAmb for *dsRNA extraction*. Mycelia were harvested from 7- to 10-day-old colonies. Fungal tissue (2.0 g) was pulverised in liquid nitrogen and nucleic acid was separated by centrifugation after addition of extraction buffer and phenol/chloroform/isoamyl alcohol (5:1:1:1) (HILLMAN / SHAPIRA / NUSS 1990). The dsRNA was purified using the cellulose column method described by MORRIS / DODDS (1979), precipitated with 2.5 volumes of ethanol and re-suspended in 1 x STE buffer (10 x STE: 100mM NaCl, 50mM Tris, 1mM EDTA). Bands were separated and analysed by electrophoresis on agarose gels (1%).

All the dsRNA obtained by the examined strains was tested in preliminary *hybridisation tests*. A probe from dsRNA of an American EP 747 hypovirulent strain was used. A digoxigenin and a non radioactive DNA labelling and detection kit (Boehringer Mannheim Germany) was adopted for the test, while the blotting of dsRNA was performed on a Zeta-Probe membrane, prehybridized, hybridised and washed by using the protocol described in the Zeta-Probe instruction manual (Bio-Rad).

A biological method to reduce the damage caused by the 'Ink disease' and based on the organic manure able to induce the re-growing and increase the development of the roots was assayed in some orchards and stands in the Mugello Area (Tuscany - Italy).

3 RESULTS
REZULTATI

The majority of the chestnut forests and orchards located in the visited countries are growing in a partially cultivated or abandoned condition. In relation to the observations carried out in Slovenia, Hungary and Slovakia, different vegetative conditions were observed in the chestnut stands. Some chestnut trees were abandoned and in competition with other tree species, such as *Quercus petraea, Fagus sylvatica, Pinus silvestris*. In some sites of the Cèvennes Area (France), the competitive species were *Quercus ilex*, *Q. pubescens*, *Pinus halepensis* and *P. pinaster*. In Italy, the competitive species were: *Betula pendula*, *Fraxinus ornus*, *Ostrya carpinifolia*, *Q. pubescens* and *Robinia*

pseudoacacia. In *Q. pubescens* and *Q. ilex* the presence of the blight fungus, *C. parasitica*, was detected (in Italy).

Blight infections and cankers were observed in all the stands in the different countries.

Forest and abandoned orchards. In the abandoned stands, the impact of the blight on the old chestnut trees is quite evident by the frequent presence of large dead branches. These completely dried old branches are the result of old infections that started many years ago and are symptomatic of the disease severity in the past. These branches were not pruned during last years and so are still observable.

The wilting of young branches, which is characterized by the presence of the dried leaves on the killed twigs and limbs, is symptomatic of recent deadly infections caused by virulent isolates. These symptoms are easy to recognise in the green crown and constitute a practical and effective index of the *C. parasitica* virulent strains activity. Several recent dying branches, especially on larger branches, are symptomatic of a situation where the virulent infections of the blight are still predominant.

Generally, however, the mortality due to recent blight attacks was limited in almost all the visited stands, in contrast with the evident severity of the first epidemic period. Branches were still dying from the activity of *C. parasitica* cankers in only a very limited number of chestnut stands in France and Italy, often located at or under 350 m above the sea level, at the lower border of the chestnut range.

The investigations have emphasized that the incidence of the affected branches was high but the number of branches killed by the blight activity was limited , especially in the Ardèche Region (France) as in Mugello, Monte Amiata and Garfagnana (Italy). The die-back occurred mainly with weak trees, suffering from competition caused by the other species. Mortality levels on sprout and shoots produced at the base of the older trees were often observed in the stands colonized by the intrusive species.

Table 1: Phytosanitary situation of the surveyed chestnut stands in France and Italy

Preglednica 1: Zdravstveno stanje popisanih sestojev v Franciji in Italiji

Location *Lokacija*	Country *Država*	Chestnut stands *Kostanjevi sestoji*	Presence of intrusive species *Prisotnost konkurenčnih drevesnih vrst*	Presence of blight *Prisotnost kostanjevega raka*	Blighted and severe damaged trees *Rakasta in močno poškodovana drevesa*	Recovering trees *Ozdravljena drevesa*	Presence of ink disease *Prisotnost črnilovke*
Saint Felix de Pallieres	F	C		+	+		
Col de Saint Pierre	F	C		+	+		
Le Lac	F	C and HF		+		+	
Le Puech	F	C and HF		+		+	
Nojaret	F	C and HF		+	+	+	
Valmalle	F	C and HF		+		+	
Jouvernargues	F	C and HF		+	+	+	
Mas de Ranc	F	C and HF		+	+	+	
Colognac	F	HF		+		+	
Pallières	F	C and HF		+	+	+	+
Le Vernet	F	HF		+		+	
Saint Roman	F	C and HF	+	+		+	
Salides	F	HF		+		+	
Montesenario	I	C and HF		+		+	+
Camperia	I	HF	+	+		+	
Lutirano	I	C and HF		+	+	+	
Gamberaldi	I	HF	+	+		+	
Firenzuola	I	C and HF		+		+	+
Palazzuolo sul Senio	I	C and HF		+		+	+
Castagno d'Andrea	I	HF		+		+	+
Grezzano	I	C and HF	+	+		+	
Vicchio	I	C and HF		+		+	+
S. Piero a Sieve	I	HF		+		+	
Scarperia	I	C and HF	+	+		+	
Montecuccoli	I	C		+	+	+	+
San Lorenzo	I	C and HF		+		+	
Molazzana	I	C and HF	+	+		+	+
Gallicano	I	C and HF	+	+		+	+

Legend/ *Legenda*: C = Coppice/*Panjevec*; HF = High Forest/*Visok gozd*

The vigorous recovery of the foliage and the spontaneous vegetative re-growth of the trees were detected in chestnut areas of the visited countries frequently.

Different cankers caused by *C. parasitica* were observed in all the stands. Normal cankers had cracks that penetrated the wood, epicormic sprouts below the canker and abundant pycnidia production. The branches or distal stems to these cankers were killed by the advancing mycelium.

Abnormal cankers were very frequent and appeared similar to that described in Italy by BONIFACIO / TURCHETTI (1973) and TURCHETTI / MARESI (1990). Some of these infections had numerous superficial cracks in the bark and few pycnidia were produced. Other cankers were completely swollen and healed. The branches or stems infected by these cankers were still living.

Cankers showing characteristics between the two described types were detected and named as 'Intermediate cankers'. New infections, characterized by a small sunken brown-red coloured area, were undifferentiated.

No infections of *C. parasitica* were detected on the natural regeneration that appeared generally not affected by the blight in the first stage of the seedlings' development.

In two recovering stands (one in the Monte Amiata, San Lorenzo - Italy and the other in the Zala County Botfölde - Hungary), grafts were successful for the effects of biological control obtained by means of a biological wax. This result must be improved especially in Hungary where Hungarian and Italian varieties of 'marrons' have been tested.

Severe damage was caused by the chestnut ink disease in Italy. Dead trees were observed in many chestnut stands visited in Mugello, Monte Amiata and Garfagnana. In some areas, chestnut trees were constrained by the ink disease, while the blight damages were not observed. In other stands, both the diseases were present, but many killed trees were the result of the *Phythopthora* spp. activity. Some severe damage was detected in moist areas at the bottom of valleys, but other foci were also present on the slopes of the mountains without apparent relation to water circulation in soil (TURCHETTI *et al.* 2000).

In France, ink - damage was observed in the Cévennes Park and in other chestnut stands (in a new plantation also) located in the Ardèche Region.

Chestnut root rot and collar rot associated with die-back of branches, gradual decline and death of the infected trees are induced by *Phytophtora cambivora* and *P. cinnamomi*. *P. cambivora* was obtained from wood isolations in France and Italy. *P. cinnamomi* was recovered from some samples collected in France, too.

The regrowth of chestnut trees affected by ink disease was observed in Mugello Area (Tuscany - Italy) as an effect of the organic manure treatments. An increase of these treatments is desirable to improve this biological method.

Competitive species able to spread in the chestnut forests and orchards were generally not detected in the chestnut coppices. The growth of the sprouts and the density of the coppices are constraint factors for the intrusive trees.

Coppices. Blight presence was intense in the majority of the chestnut coppices visited in France, Italy and Hungary. In France, the visited coppices were abandoned for the last 20 to 30 years. In the Cévennes area (France), *C. parasitica* attacks were observed in all the examined stands and ranged between 28 and 91%, while in Mugello (Italy) this range is restricted between 62 and 87%.

The levels of mortality were 12 and 35% in the different examined plots. The percentages of killed trees in three French sites (Le Lac, Jouvernagues, Palleries) were 20, 23 and 31% respectively, while in the other stands were between 12 and 16%. High percentages of died sprouts were recovered in two Mugello sites: Grezzano (35%) and Lutirano (22%). The majority of dead sprouts were suffering from the competition among them in the same stump and from their dominated condition.

Other factors, such as natural injuries are involved in the chestnut sprouts dieback and their incidence is less than 10% (Table 2).

In the plots visited in France and Italy a consistent part of the sprouts examined was blighted, but living, and this result could be indicative of a different disease introduction in the chestnut stands of the other countries. *C. parasitica* has been present in Italy for the last fifty years and the blight pressure on Italian chestnut stands is quite evident, while in

France the disease spread later, from 1956, and the percentage of non-diseased sprouts is still high. The blight epidemic started more recently in Hungary and Slovakia.

Normal, abnormal and intermediate infections were present together in the blighted stands examined in the different European countries. In some stands visited in France, as in the Valmalle site, normal cankers prevailed (30%), but the percentage of killed sprouts was low (12%). The reason, probably, was due to the large diameters of the sprouts. In the Grezzano stand (Italy), the dieback level was high (35%) and the predominance of normal attacks (40%) was confirmed (Table 2).

Table 2: Incidence of diseases in chestnut sprouts observed in plots located in France and Italy

Preglednica 2: Znaki bolezni na kostanjevih poganjkih na ploskvah v Franciji in Italiji

N	Locations *Lokacija*	Country *Država*	N° plots *Št. ploskev*	Non-diseased sprouts *Neokuženi poganjki*		Blighted living sprouts *Živi rakasti poganjki*		Sprouts died from: *Poganjki odmrli zaradi:*					
								Blight *Kostanjev rak*		Ink *Črnilovka*		Other factors *Drugi dejavniki*	
				n	%	n	%	n	%	n	%	n	%
1	Col de Saint Pierre	F	1	203	65	59	19	49	16				
2	Le Lac	F	1	125	48	80	31	53	20			3	1
3	Saint Roman de Tousque	F	1	60	44	28	21	17	13	10	7	21	15
4	Le Puech	F	1	52	38	60	44	18	13			8	5
5	Nojaret	F	1	43	33	57	43	17	12			15	12
6	Valmalle	F	1	64	59	18	16	13	12			14	13
7	Jouvernagues	F	1	21	14	81	51	37	23			18	12
8	Mas de Ranc	F	2	70	44	67	42	19	12			3	2
9	Saint Felix de Palleries	F	1	81	45	59	33	26	14	7	4	8	4
10	Palleries	F	1	5	5	73	60	38	31			5	4
11	Montesenario	I	2	43	9	358	69	98	18	4	1	16	3
12	Lutirano	I	1	60	21	128	45	62	22	10	4	23	8
13	Firenzuola	I	1	128	22	324	56	98	17	15	3	8	2
14	Palazzolo sul Senio	I	1	65	22	198	66	37	12				
15	Grezzano	I	1	60	30	53	27	72	35	3	1	14	7
16	Vicchio	I	1	28	21	82	62	22	17				
17	Montecuccoli	I	1	78	32	125	52	38	16				
18	Scarperia	I	1	37	17	173	74	22	9				
19	San Lorenzo	I	1	58	13	380	83	15	3	2	1		
20	Molazzana	I	1	52	15	256	75	26	8	5	2		

The abnormal infections were predominant in all the chestnut coppices visited in the European countries. Intermediate infections were also present in all examined stands.

Infections were observed at all levels of the stem and branches and the size of the cankers was different. Some of them extended along the trunks and had completely encircled the stems without producing dieback of the sprouts.

Relationships between diameter and mortality were observed. Most of the sprouts killed by the disease belonged to the diametric classes of 5 and 10 cm. The percentages of mortality decreased in correspondence to size of the stems and disappeared at the largest diameters (Figure 1). The minor diameter classes corresponded to dominant or subdominant sprouts present in most coppices of the visited stands. This relationship was also observed in Slovenia and data on this subject are being collected.

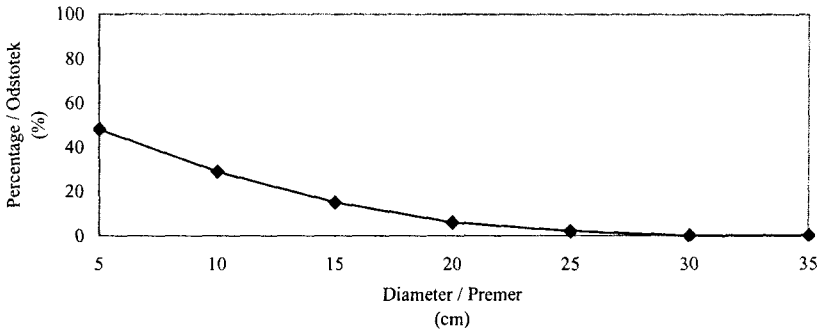

Figure 1: Influence of sprout diameter on the blight dieback
Slika 1: Vpliv premera poganjka na delež odmrlih poganjkov

In a chestnut forest in Monte Amiata, the dead sprouts which died from blight were removed, while the others, non-diseased or affected by healing cankers, were released. This treatment has decreased the inoculum level of the more injurious isolates of *C. parasitica* and will improve the hypovirulence spread, so that after five years, the remaining sprouts will have been all living and developing.

Ink disease was observed in some coppices of Italy and France. In the Mugello regions, new ink damage was observed in several stands and the complete destruction of large coppices was observed. *P. cambivora* was isolated from samples collected in the infected plot. In a nursery, *P. cinnamomi* was obtained from diseased and dead chestnut seedlings.

Laboratory: The following cultures were obtained from the isolations tried on the different canker samples collected in the different European countries:

a) virulent or normal with mycelia yellow-orange and abundant orange pycnidia,
b) hypovirulent with white - orange or completely white mycelia. Pycnidia are absent or few with large dimension,
c) intermediate with intermediate morphological characters of the cultures.

These differences were observed in the morphological characters of the cultures when the isolates were grown on PDAmb and on chestnut stems placed in Petri dishes containing PDAmb. On this medium, the hypovirulent strains, unable to sporulate, produced pycnidia after one month.

Tests for phenol-oxidase activity have emphasized the different abilities of the virulent and hypovirulent strains to produce this enzyme. Some hypovirulent strains were unable to form a brown area in the medium around the mycelia inoculum, while the production was detected for the virulent strains.

In relation to the isolates collected and tested, vegetative compatibility tests have emphasized that some V - C (vegetative-compatibility) groups (two groups) are common in different European countries, while others are specific and spreading in each country (Table 3) and new V-C groups were identified, too.

DsRNA assays pointed out that there were no differences between the electrophoretic profiles of strains coming from the different Italian regions and from the other visited countries: all the assayed hypovirulent strains were characterized by the constant presence of a L-dsRNA (Large dsRNA, of about 12 kb, sometimes related to other bands (Small and Medium dsRNA) .

The hybridisation tests confirm a strong L-dsRNA homology and suggest the presence of one or few types of dsRNA in the natural hypovirulent strains observed in the investigated woods (HILLMANN *et al.* 1995).

Table 3: Representative laboratory results on the *C. parasitica* strains isolated from the visited European chestnut stands.

Preglednica 3: Nekateri laboratorijski rezultati sevov C. parasitica, izoliranih v popisanih kostanjevih sestojih

Country *Država*	Visited sites *Ploskev*	Strains total *Sevov skupaj*	Morphology of the cultures *Morfologija kultur*			Bavendamm test positive *Bavendammov test pozitiven*			V-C Groups *V-C skupine*	New V- C groups *Nove V-C skupine*	dsRNA presence *Prisotnost dsRNA*
			N	H	I	N	H	I			
France *Francija*	Park de Cevennes	40	15	12	13	15	5	10	2, 5, 8, 10	3	10
Italy *Italija*	Mugello	15	7	5	3	7	1	3	1, 3, 5, 8	1	6
	M. Amiata	25	9	7	9	9	2	9	4, 6, 8, 10	2	7
	Garfagnana	15	3	6	6	3	2	6	2, 5	1	6
Hungary *Madžarska*	Zala County	20	9	4	7	11	2	6	1, 3, 5,	1	4
Slovenia *Slovenija*	Haloze Ravnica	15	3	7	5	4	3	4	2, 6	1	7
Slovakia *Slovaška*	Slanec	7	3	2	4	3		2	3, 8		2

4 DISCUSSION
RAZPRAVA

Chestnut blight is a major widespread disease in European chestnut woods. The ink disease produces severe damage in these chestnut forests, but it appears only in restricted areas in France, while in Italy it is spreading again.

The abandonment of many chestnut orchards in the visited countries and their natural conversion in to chestnut forests, could have increased the spread and the incidence of *C. parasitica* attacks in the past and now.

Another aspect is the vegetative condition of many chestnut trees that are suffering from competition. The contribution of nutrients produced by the decomposition of the chestnut

foliage is often utilized by other species spontaneously growing in the same chestnut stand. The gradual degradation of chestnut forests placed in unfavourable sites is occurring and other species (*Quercus pubescens* and *Robinia pseudoacacia* in Italy, *Quercus pubescens*, *Q. ilex* and *Pinus pinaster* in France) are developing in these chestnut stands. Trees of these different species are competing against chestnut trees as a consequence of the abandonment.

In this situation some diseases such as the ink disease can increase and speed the gradual evolution of these stands, killing the chestnut trees more often than other species.

The visited chestnut coppices are in more favourable vegetative condition (regarding density) and so the competitive species are often unable to colonise these woods.

C. parasitica may be now considered to be a naturalised component of the chestnut ecosystems, where its role may be considered as a bio-regulation factor. The severity of the damage and the intensity of the disease seem to be related to environmental conditions and the effectiveness of management. Dominated, weakly or stressed trees or sprouts without any chance of survival are easily colonised and killed by chestnut blight fungus (AMORINI *et al.* 2001) while vigorous trees can recover and also produce fruit in the presence of disease, but only if hypovirulence is dominant. In fact, a massive predominance of the abnormal cankers was observed in most of the visited forests, while normal infections were predominant only in a few limited areas. Widespread hypovirulence has greatly reduced the severity of the blight. The re-growth of the chestnut trees observed in Italy, France, Slovenia, Slovakia and Hungary and the limited damage caused by the blight in Slovenia and Hungary emphasises the effectiveness of the hypovirulence on fungus behaviour almost everywhere.

This favourable trend can be diminished when other factors of stress or disturbance or unfavourable site conditions are involved in the dynamics of these chestnut stands. More detailed investigations on this topic must be performed.

The observed situation can be considered as a consequence of the relative homogeneity in the virulent fungal population (TURCHETTI 1994, CORTESI / MILGROOM / BISIACH 1996) and by the ability of the hypovirus to spread between strains in natural

conditions (MARESI *et al.* 1995). However, the occurrence of new genotypes was detected during this investigation and this result is symptomatic of a continuous evolutionary process in the pathogen population. Further investigations are desirable to test the pathogenicity and the virulence of these new lines of *C. parasitica*. For this, preliminary observations carried out in the stands, have shown that the level of damage caused by these new genotypes was still unremarkable. Furthermore, the absence of severe damage suggests a good ability of the hypovirulence factors in spreading and maintaining their prevalence in a changing population of the pathogen.

Besides the appearance of new genotypes, the predominance of the hypovirulence can also be altered by the action of stress factors, such as drought, fire and frost. Relationships between these factors and severe damage observed in some new foci of blight need to be verified.

In relation to the visited chestnut stands and coppices in European countries, it is possible to predict an increase of the blight's presence in Europe, but the presence and the natural spread of the hypovirulence could decrease the damage caused by *C. parasitica*.

The availability of a sustainable and effective natural biological control encourages the interest in chestnut forests and assures greater likelihood of checking the blight in the future. From this perspective, the abandonment of chestnut wood management, due to economic factors or to the social evolution of various Mediterranean mountain areas, could be considered the main cause of chestnut wood's decline.

The abandonment could directly influence the evolution of some chestnut orchards and forests towards mixed woods where chestnut presence is reduced because of competition with other species. At the border of the natural range of *C. sativa*, the interspecies competition could cause the complete disappearance of chestnut, while it could maintain predominance in the central area of its range. Blight is favoured by the absence of pruning and cutting but the predominance of hypovirulence can reduce the damage caused by the disease on trees, limiting the mortality to the plants which suffer from greater competition.

Chestnut coppices could evolve into chestnut forests with a strong competition between sprouts of the same stumps, but with few chances of introduction for different species. In this case blight can play a positive role, speeding up the death of the weakly and dominated sprouts and becoming a positive factor of stand evolution.

Where the management of chestnut orchards and chestnut coppices is still economically possible, the target of the cultural practises would be the maintenance of hypovirulence on trees and coppices (SANTAGADA / MARESI / TURCHETTI 1996), while the competition of other species would be reduced and checked by cuttings. Simple management could preserve a lot of chestnut orchards or forests that are very interesting for tourist and landscape purposes.

The presence of the ink disease could increase and speed up the evolution of some chestnut forests towards mixed woods, where chestnut can completely disappear and be replaced by other species less susceptible to the parasite. Here, the presence of *P. cinnamomi*, more polyphagus than *P. cambivora*, could increase new severe damage, directly threatening the survival of these woods and opening ecological and soil-conservation problems (TURCHETTI / PARRINI 1993). Further investigations are planned on these parasites and on the environmental factors that could improve the spread of this disease.

5 POVZETEK

Opravili smo popise zdravstvenega stanja kostanjevih sestojev v Franciji, Italiji, Sloveniji, na Madžarskem in Slovaškem. Kostanjev rak je najpogostejša bolezen v teh sestojih. V preteklosti je Cryphonectria parasitica povzročala močne poškodbe v kostanjevih sestojih v Franciji in Italiji. Vendar je v večini pregledanih sestojev prišlo do ponovnega odganjanja dreves, ki jih je v preteklosti okužil kostanjev rak. V Sloveniji, na Madžarskem in Slovaškem se je bolezen razširila šele pred kratkim. Na mnogih izmed pregledanih ploskev so bila prisotna ozdravljena rakasta drevesa in hipovirulentni sevi parazita, čemur smo pripisali zmanjšan obseg poškodb. V opuščenih sestojih se pojavlja večje število drevesnih vrst, ki konkurirajo kostanju. Črnilovko pravega kostanja, ki jo večinoma povzroča Phythophthora cambivora, smo opazili v sestojih v Franciji in Italiji;

poškodbe, ki jih povzroča, so zelo učinkovite, njihova pogostnost pa narašča. V prispevku obravnavamo vpliv obeh bolezni na razvoj kostanjevih sestojev in na trajnostno gospodarjenje z njimi.

V kostanjevih nasadih in panjevcih, kjer je gospodarjenje še ekonomično, je cilj vzdrževati hipovirulentne osebke kostanja, z ukrepi pa uravnavati prisotnost konkurenčnih drevesnih vrst.

Prisotnost črnilovke pravega kostanja pospešuje prehod čistih kostanjevih sestojev v mešane sestoje, v katerih kostanj postopoma izgine in ga nadomestijo druge, na črnilovko pravega kostanja bolj odporne drevesne vrste.

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