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BIOLOGICAL CONTROL OF CHESTNUT BLIGHT IN EUROPE

Ursula Heiniger and Daniel Rigling
Swiss Federal Institute of Forest, Snow and Landscape Research, CH-8903 Birmensdorf, Switzerland

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INTRODUCTION

Cryphonectria parasitica (Murr.) Barr. (Syn. *Endothia parasitica* (Murr.) And. & And.), the causal agent of chestnut blight, is a fungal pathogen of *Castanea* and *Quercus* species. After its introduction at the beginning of this century, *C. parasitica* virtually eliminated *C. dentata* Borkh. as an important forest tree species in the eastern United States. A similar result was anticipated when chestnut blight was found in Europe, but the unexpected appearance of transmissible hypovirulence—a unique natural biocontrol phenomenon—prevented the European chestnut (*C. sativa* Mill.) from succumbing to the blight (for reviews see 5, 51, 69, 92, 101, 103, 130).

Castanea sativa was introduced by the Romans from Minor Asia (79). This tree species was formerly of major economic importance in the mountainous areas of Southern Europe, in the southern foothills of the Alps from Italy into Hungary, and along the Black Sea. It provided timber, firewood, tannin, and litter bed on marginal land, and produced nuts for human consumption and animal forage. Chestnuts are traditionally grown as coppices with a rotation period of 15 to 30 years, as coppices with standards, in high forests, and in orchards. The latter are often formed of old-growth giant trees (100 years is not uncommon), grafted with the local varieties (Figure 1). Socioeconomic changes and the introduction of the devastating chestnut blight (*C. parasitica*) caused a rapid decline in chestnut cultivation in many regions after the second world war (54, 76).

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Natural dissemination of hypovirulence has allowed many chestnut stands to recover. Renewed interest in this tree species and the economic importance of its nuts (3) led to a revival of chestnut cultivation in Europe. Selected nut-producing varieties of *C. sativa* are now cultivated in plantations.

This review concentrates on the epidemic of chestnut blight in Europe, the biology of hypovirulence, and its effect on the phytosanitary situation in European chestnut stands.

THE CHESTNUT BLIGHT EPIDEMIC IN EUROPE

The Dissemination of Chestnut Blight

C. parasitica, a wound parasite, infects branches and stems of *C. sativa*, causing the smooth bark of young branches to become reddish and sunken (Figure 2). The fungus grows in the cambium and in bark tissue forming pale brown mycelial fans. Plant reaction results in bark cankers. Girdling of branches by the fungus induces wilting and dieback of the distal parts. Pycnidia and perithecia, embedded in an orange-yellow stroma, break through the bark. The disease is readily visible from the dry leaves that remain on the twigs and from the copious epicormic shoots produced below the cankers. The roots are not infected and stumps will sprout again.

Chestnut blight was officially recorded in Europe in 1938, near Genova, Italy (20). The area of blight was already extensive (48) and the disease was soon observed throughout Italy (15). The disease spread rapidly and by 1948, 5–100% of the trees chestnut stands in north-west Italy were affected by blight (47). Del Guerra (47) claims that the blight had been introduced much earlier but had gone unnoticed because of the widespread decline of Italian chestnut stands caused by ink disease and general neglect[1] *Castanea crenata,* the Japanese chestnut that is resistant to ink disease, was planted in Italy (48), Spain, and France (43) during the 1920s. *C. parasitica* is a weak pathogen on Asian chestnuts and was probably imported into North America with Asian chestnut plants (5). Similarly, imported Asian chestnut plants may have been the source of the epidemic of chestnut blight in Europe. This hypothesis is suggested by the fact that, in Spain, chestnut blight was first noted in 1947 on *C. crenata* (22) in a stand of apparently healthy *C. sativa.* The cankers dated back at least 15 years (106). In addition, over 25,000 plants and scions of *C. mollissima* and hybrids (*C. mollissima* x *C. dentata* and *C. mollissima* x *C. sativa*) were imported from the USA into Italy between 1950 and 1953 in the

[1]Ink disease, caused by *Phytophthora cambivora* (Petri) Buism., attacks the roots of chestnuts, leads to branch mortality, symptoms of general decline, and finally to the death of the plants.

search for blight-resistant chestnuts (2, 27). Whether these imports resulted in additional introductions of *C. parasitica* is not known.

From Italy the disease spread into the adjacent countries. Chestnut blight probably also went unnoticed for a long time in France because of decline due to ink disease. Severe frost mortality in 1956 further hindered the identification of the blight (43). From 1950 to 1975 the disease spread from Italy through the former Yugoslavia to the Albanian border (71).

By 1967 most chestnut-growing areas in Europe were infected with *C. parasitica* (Table 1). New infection foci had recently been noted in orchard plantations in Portugal (A Carvalho & CAG Abreu, personal communication), in Switzerland north of the Alps (77), in Germany (118), on Mount Athos in Greece (49), and in the departments Indre and Isère in France (C Jousselin, personal communication).

Introduction of the disease often went unnoticed until branch mortality became obvious. Expansion of the disease is currently slow because there are no continuous chestnut stands in Middle Europe. It is hoped that the scattered stands in northern Europe and Great Britain will remain blight-free. Long-distance transport of *C. parasitica* is not fully understood. Ascospores and mycelial particles can be carried by wind, birds, and insects, and people are an important vector in the transport of chestnut plants and wood. Nutshells can also be infected (82). Infected logs of host trees (i.e. Castanea and *Quercus*) harbors fungal mycelium, pycnidia, and perithecia. Grafted plants often develop cankers and may be a source of inoculum. Additionally, spores may adhere to the glandulous surface of young plants. Symptomless twigs of *C. sativa* may even be colonized by the fungus, as was recently demonstrated (33).

The European and Mediterranean Plant Protection Organization (EPPO) considers *C. parasitica* to be an A2 quarantine organism and recommends debarking of *Castanea* and *Quercus* wood and importation of wood in bark or plants for planting only from disease-free regions (122).

Disease Intensity at the Beginning of the Epidemic

Attacks of *C. parasitica* were at first severe and resulted in high mortality. In coppice stands 90% of the sprouts were infected by the age of 4–5 yr and died within a few years (26), but the root stocks survived and resprouted. In old-growth chestnut orchards the entire crown of the trees became blighted. Ten to fifteen years later the orchard trees declined and died within six years, without having been able to regenerate sprouts (26) because of root rots (J Schüepp, personal communication). As a result, some chestnut orchards were totally destroyed (64). Once the disease reached a new orchard, the incidence of disease increased rapidly, as shown by a survey in the Ticino: within two years the number of blighted trees rose from 14% to 65% (117).

However, reports from North America led many authors to conclude that the severity of disease was lower in Europe and its dissemination slower (16, 24, 48, 117, 119).

HYPOVIRULENCE

In 1951, Biraghi (24) discovered a chestnut coppice that looked "surprisingly healthy" despite infection in 85% of the shoots. Some cankers were very long and the bark was slightly cracked and dark in color. The fungal mycelium was restricted to the outer layers of the bark, resulting in superficial canker development (Figure 3). The healing and nonlethal cankers were comparable to cankers found on *C. crenata* in 1949 (23). Biraghi ascribed this development to increased resistance of the trees that was mainly attributable to repeated cutback of the sprouts (24). In 1964, Grente (65) isolated atypical strains of *C. parasitica* from healing cankers near Como, northern Italy. Pigmentation and sporulation were lower in these strains than in normal isolates of the pathogen, giving them a white appearance when grown on potato dextrose agar (PDA). The atypical strains were reduced in virulence when inoculated in chestnut trees. Moreover, when white strains were coinoculated with normal isolates most of the resulting cankers healed. Grente (65) called this phenomenon *hypovirulence*. The white, as well as the hypovirulent, phenotype of the fungus is cytoplasmically controlled (68, 131) and associated with high molecular weight double-stranded (ds) RNAs (44). Hypovirulent strains can convert virulent strains to hypovirulence by the transfer of dsRNA via hyphal anastomosis (4, 10, 112; Figure 5a). This phenomenon is the basis for biocontrol.

Viral dsRNA, The Causal Agent of Hypovirulence

White strains containing similar-sized dsRNA were found in various European countries, including France (44), Italy (44, 58, 60), Switzerland (112), Croatia (111), Greece (136), and Germany (D Rigling, unpublished data; Table 1). Hybridization experiments indicated close similarity between dsRNAs from Italian and French hypovirulent strains (78, 88). The dsRNAs derived from a French hypovirulent strain have been characterized in great detail and recently reviewed (103). They consist of one large dsRNA (L-dsRNA) that contains two continous coding domains (ORFA and ORFB) and multiple defective interfering segments (120, 121). Genetic organization and expression, as well as the replication strategy of the L-dsRNA, strongly suggest a viral origin of the dsRNA (40, 41, 53, 121). The dsRNA is not encapsidated but is associated with fungal membrane vesicles (72, 102). The introduction of L-dsRNA into *C. parasitica* via DNA-mediated transformation established that the dsRNA is indeed the causal agent of hypovirulence (38). The defective dsRNAs are internally deleted forms of the L-dsRNA (121), which are generated upon

subculturing of dsRNA-containing strains (4, 10, 120), transfer of the L-dsRNA via hyphal anastomosis (38), and after passage of the fungus through host tissue (36). The contribution of the defective interfering dsRNAs to the expression of the hypovirulent phenotype is not clear. The dynamic competition for replication factors between defective and genomic dsRNAs could have a significant effect on phenotype expression, as outlined by Shapira et al (121). Chen et al (36), however, found no phenotypic changes associated with the appearance of defective dsRNAs.

The origin of viral dsRNA is unknown and is the subject of much speculation (52). The hypovirulence-associated dsRNA of *C. parasitica* is related more closely to plant potyviruses than to known fungal dsRNA viruses (84). dsRNA-containing strains of *C. parasitica* were most likely introduced from Asia, together with normal strains, since dsRNA has been detected in hypovirulent strains of *C. parasitica* from China (89). However, its relatedness to the European type dsRNA has not yet been determined.

Transmission of dsRNA

dsRNA is transmitted at variable frequencies into asexually produced conidia (115, 128). The dsRNA-containing strains are female-sterile but can serve as male partners in sexual crosses (6). dsRNA, however, is not transmitted into the ascospores (6, 36, 137). Transmission of dsRNA via hyphal anastomosis is under control of a vegetative compatibility (v-c) system involving 5-7 v-c loci (reviewed in ref. 6). If identical alleles are present at all v-c loci, viable anastomoses are formed and dsRNA is rapidly transmitted between colonies (10, 94). In contrast to dsRNA, mitochondrial DNA does not readily move between vegetative-compatible strains (61). However, transfer of dsRNA can also occur between strains in different v-c groups, but more slowly and at lower rates (10, 86). In these cases, temporal anastomoses might allow the dsRNA to pass before the incompatibility reaction kills the fused cells.

Hypovirulence Expression of dsRNA-containing Strains

Wide variation has been observed in the expression of hypovirulence-associated phenotypes in dsRNA-containing strains. The typical white appearance of dsRNA-containing strains on PDA has been widely used to distinguish between virulent and hypovirulent strains of *C. parasitica*. However, several studies suggest that no clear relationship exists between the white cultural phenotype and hypovirulence. In a virulence study conducted by Bazzigher (17), several white strains produced similar-sized lesions as normal, virulent strains in 3 months. Within 15 months the white strains killed about 50% of the five-year-old seedlings and the normal strains killed 80–100%. In this study, host mortality was the main parameter distinguishing white strains from normal strains. Pennisi & Minvervini (107) reported that strains with "hypo-

virulent" culture morphology are either hypovirulent or intermediate virulent when compared to normal strains. A continuum in virulence expression ranging from avirulent to almost normal virulent has been observed in dsRNA-containing strains (50, 60, 112, 126). Some dsRNA-containing strains required field tests spanning more than one growing season for their virulence deficiencies to be detectable (52). The expression of different levels of hypovirulence appears to be determined by cytoplasmically transmitted dsRNAs and is independent of the nuclear genetic background of the recipient strains (52, 112).

Molecular Basis of Hypovirulence

Typically, the presence of the dsRNA does not greatly reduce vegetative growth of the fungus either on solid (9) or in liquid culture media (19, 113; Figure 5). Hebard et al (74) reported that the formation of mycelial fans, an important step in virulence expression, is reduced in hypovirulent strains. Molecular analysis indicated that dsRNA reduces the accumulation of specific mRNAs and polypeptides (108, 109). Accumulation of the metabolite oxalate (73) and of several proteins is reduced in dsRNA-containing strains of *C. parasitica.* These proteins include an extra- and intracellular laccase (112, 114), a cell-surface protein (35), a cutinase (132), and a putative mating-type pheromone (141). Subsequent studies on the biosynthesis of the extracellular laccase demonstrated that down-regulation of this enzyme by dsRNA occurs at the level of transcription or stability of laccase mRNA (37, 113). Recent reports suggest that dsRNA perturbs cellular-signaling processes that normally result in laccase induction (87). Whether oxalate or any of the proteins mentioned above play a role in the pathogenicity of *C. parasitica* is as yet undetermined. Expression of a specific viral coding domain (ORFA) is responsible for the reduction of pigmentation, sporulation, and laccase accumulation of the fungus but not for hypovirulence (39).

DEVELOPMENT OF NATURAL HYPOVIRULENCE IN EUROPE

The phenomenon of natural hypovirulence of chestnut blight in Europe is characterized by a decrease in the severity of disease and by the presence of white hypovirulent isolates of *C. parasitica.*

Biraghi first observed this phenomenon in 1951. He concluded from analysis of cross-sections of cankered tissue that the healing process must have started 4–5 yr earlier (24). In 1954, he reported the presence of healing cankers "in many parts of Italy where the pathogen has been present for a certain number of years" (25).

In 1964, Grente (65) isolated white strains of *C. parasitica,* the causal agent the hypovirulence, from healing cankers, found in Como, Italy, and in three areas in France (66). The frequency of healing cankers and the recovery of

Table 1 The chestnut blight epidemic in Europe

Country	Cp[a]	H[b]	W[c]	dsRNA[d]	VCGs[e]	P[f]	Reference[g]
Italy	1938	1951	1964	+	>17	+	20, 21, 24, 44
Spain	1947	1992	1992	[i]	>5	−	106, 98
Switzerland	1948	1975	1975	+	>6	+	14, 18, 30, 32, 112
Croatia	1950	1978	1981	+	8	+	70, 71, 85
France	1956	1964	1964	+	>20	+	43, 65, 68
Greece	1964	1975	1984	+	2	ND	134, 136,
Hungary	1965	ND[h]	−	−	−	ND	
Turkey	1967	ND	−	[i]	−	+	45, 46
Albania	1967	1083	1984			ND	
Austria	1970	1993	1993		>7	+	55
Slovakia	1976	ND			2	+	83
Portugal	1989	ND	−				
Germany	1992	1993	1993	+	2	ND	118

[a] Year of first observation of *Cryphonectria parasitica*
[b] Year of first observation of healing cankers
[c] Year of first isolation of white (hypovirulent) *C. parasitica* strains
[d] +, dsRNA detected
[e] Number of v-c groups
[f] Perithecia: +, present in the field
[g] Informations were also obtained by personal communications from Croatia, M Halambek; Greece, S Xenopolous & S Diamandis; Hungary, P Szentivanity; Albania, A Shyti & P Carçani; Austria, E Donaubauer and E Waindinger-Wilhelm; Slovakia, G Juhasova; Portugal, A Carvalho Olivierea & CAG Abreu; Germany, D Seemann & J Zajonc.
[h] ND, not detected
[i] no information

white isolates was much lower in France than in Italy. Subsequently, white strains of *C. parasitica* were found to be widespread throughout Europe, including some relatively isolated regions (e.g. Greece, Sicily, Spain) far away from northern Italy (Table 1).

No clear picture about the development of hypovirulence can be drawn from the data available. As the initial blight epidemic, the occurrence of hypovirulence went undetected for a long time. Shain (119) suggested that the spread of hypovirulence in Europe lagged only slightly behind the original blight epidemic, a view supported by a study in Germany, where chestnut blight was recently discovered in two chestnut stands in Baden-Württemberg (118). Analysis of tree-growth rings indicates that blight was introduced in 1985. In 1992, one white, dsRNA-containing isolate was found among 170 normal isolates, all belonging to the same v-c group (J Zajonc, D Rigling, D Seemann, unpublished results). In contrast, no white strains were detected in several heavily infected chestnut stands in northern Switzerland. However, the sampling size at each site was much smaller (30, 31). Studies at the front of the epidemic of chestnut blight may give more quantitative information about the time of appearance and the dissemination of hypovirulent strains in Europe.

HYPOVIRULENCE AND DISEASE DEVELOPMENT

Although hypovirulence spread rapidly into most chestnut-growing areas, the virulent form of the chestnut blight fungus did not disappear. Actively growing cankers are found everywhere and lead to death of branches and stump sprouts. Compared to reports from the beginning of the epidemic, the incidence of disease is still high but its severity has been drastically reduced. Reports from Italy and the Ticino in southern Switzerland, a region adjacent to northern Italy with comparable chestnut stands and a similar incidence of disease, corroborate this development.

CANKER DEVELOPMENT In 1947, when the blight was considered severe, canker-growth rates of 16 cm/yr were reported from Italy (15). This compares to canker-growth rates of 11–16 (average = 12.5) cm/yr measured in the Ticino in 1959 (117). In a recent study (30; M Bissegger, unpublished data) an average canker-growth rate of 7.9 cm/yr was measured initially, dropping to 2.5 within two years. Similarly, the proportion of cankers that grew more than 19 cm/yr fell from 7.6% to 1.7% within the same period and the proportion of nongrowing cankers rose from 19% to 74%. Nongrowing cankers were first observed in 1959 and their proportion rose from 10% to 19% within a year (J Schüepp, unpublished data). This fact implies that hypovirulence was probably already present but had not been noted.

INCIDENCE OF DISEASE The percentage of cankered trees remains high. Surveys in 1963 (before the recognition of hypovirulence in 1976) (16) and in 1988 (42) showed that the same percentage of chestnut trees were blighted in the Ticino, i.e. 58% and 55%, respectively. In 1959, the incidence of disease varied greatly in specific chestnut stands and increased steadily by 10 to 20% per yr (117). Today, with hypovirulence present, the proportion of infected sprouts of two six-year-old coppice stands rose from 37% to 55% within two years (75). These numbers compare well with a survey from Italy where the proportion of cankered shoots increased from 39% to 61% over the same time span (138).

MORTALITY With hypovirulence present, the mortality of trees and sprouts seems to be reduced. However, the data are difficult to evaluate as the numbers of dead trees represent not only the intensity of the disease but rather the age of the stand and the effectiveness of its management. The proportion of dead trees at present ranges from zero in a managed coppice stand to 10% in an abandoned stand in the Ticino (42). In Italy, a rate of 20% dead sprouts was counted in a young coppice stand (125). At the beginning of a rotation, the number of dead stump sprouts rises rapidly, for example from 12% to 23% in

two years in an orchard in Italy (140). Likewise, 17% of all sprouts died within two years in two Swiss clear-cut coppices, although only about half of the mortality was attributed to chestnut blight (75), the rest to competition-killing. Mortality caused by *C. parasitica* and competition was significantly higher for the thinner sprouts (30), which is in accordance with the finding that blight susceptibility of *C. sativa* decreases with increasing diameter of the sprouts (17). *C. parasitica,* together with other causes, results in natural thinning of dense coppice stands, which often have more than 10 sprouts per stump.

DISEASE SEVERITY Hypovirulence has greatly reduced the severity of the disease. Superficial and healing cankers are widespread and do not cause mortality in the host. However, the frequency of active and healing cankers varies widely between stands, even within the same region. Investigations in seven communities near Naples, in southern Italy, revealed cankers on 25–92% of the trees (142). Depending on the location, 5–72% of these cankers were assessed as healing cankers. Similarly, in Calabria and Sicily about 50% of the shoots had cankers, of which 38% were healing (107). In the Ticino, 95% of the cankers were described as healing (42); in the Viterbo area, 30–80% (91); in several other Italian areas, 14–22% (28), whereas in the South Tyrol (Italy), less than 10% healing cankers were observed (129).

DISEASE DEVELOPMENT Active cankers are thought to be prevalent in the beginning of a disease cycle, then healing and healed cankers increase as a result of the spread of the viral dsRNA in the fungal population.

This dynamic in the development of disease is shown in studies in clear-cut coppices, where the young sprouts gradually become infected. Mutto & Del Sole (99) observed an increase in the number of active cankers up to four years after clear-cutting. The canker-growth rate was high and resulted in mortality. With age of the sprouts and increased manifestation of hypovirulence, mortality of sprouts was reduced and the number of healing and healed cankers rose.

HEALING CANKERS AND WHITE ISOLATES OF *C. parasitica* Several studies showed that white isolates of *C. parasitica* were preferentially associated with healing and healed cankers (60, 123, 140). The average growth rate of cankers from which white isolates were obtained was significantly slower than the growth rate of cankers with normal isolates (32). Nongrowing cankers yielded 42% white, 16% normal, and 42% no isolates of *C. parasitica* (75). Surprisingly, some white strains were as virulent as normal strains in field tests (30).

The proportion of normal and white isolates of *C. parasitica* sampled at a site or in a region only approximately represents the severity of blight. For instance, in the region of Viterbo, where 59% of the cankers were healing, 61% white or intermediate isolates were obtained, but considerable variability

existed between the five sites investigated (91). In Sicily, with 28% healing or healed cankers, more white isolates were obtained than in Calabria, with 38% healing and healed cankers (107).

Cankers probably often result from multiple infections and normal and white strains add to the dynamic of canker development. Repeated sampling of identical cankers revealed a slight increase in the frequency of white isolates (2–3% within two years). Some white and normal isolates obtained from long cankers belonged to different v-c groups (66; H Cattelan & U Heiniger, unpublished data), which indicates that long cankers may be the result of multiple infections. It is also conceivable that normal strains of *C. parasitica*, which belong to an incompatible v-c group or are not yet fully converted, may be present within a healing canker.

Dissemination of hypovirulence

How the viral dsRNA spreads within the fungal population is unclear. The dsRNA has not been found in ascospores (6, 36), which suggests that these spores are not important for dissemination of hypovirulence. Conidia containing dsRNA are probably the main source for dissemination of the virus. Conidiation, however, is low on healing cankers. Carriers like insects and mites, which move between cankers, might be involved in the transport of dsRNA-containing conidia (100, 116). Grente (66) suggested that hypovirulent strains could also be spread in tiny chips of infected bark that are disseminated by wind, insects, birds, or mammals.

Vegetative Compatibility

Transmission of hypovirulence in *C. parasitica* is restricted by vegetative incompatibility, as shown both in vitro (10; Figure 5b) and in vivo (13). High v-c group diversity in relatively small areas is thought to be the main reason for poor dissemination of hypovirulent strains in North America (5).

The number of v-c groups is limited in every European country (Table 1). In an analysis of strains from several Italian and French regions, Anagnostakis et al (11) found 33 different v-c groups. This number is much lower for specific regions or sites, ranging within Italy from 11 in Calabria (107) to 4 in Modena (137). The v-c group diversity in a single chestnut stand is usually very limited and can be as low as one per site (66). Most isolates were often assigned to one or two dominant v-c groups (18, 63, 107). Several infection sites with only one v-c group have been reported at the front of the epidemic of blight in Europe (31, 135; D Seemann & J Zajonic, personal communication).

In addition to low v-c group diversity, spread of viral dsRNA in Europe was probably further facilitated by hypovirulent isolates with broad conversion capacity (18, 58, 81, 127). Such strains convert virulent strains from different

v-c groups (86). The limited number of v-c groups found in Europe contrasts with North America where 67 v-c groups were identified in Connecticut alone (12). In West Virginia (96) and Connecticut (12), up to 27 and 48 different v-c groups, respectively, were reported in single, small clear-cut plots . Pronounced founder effects were observed at the beginning of an infection cycle in the US, followed by a rapid increase of v-c group diversity (8, 12). In contrast to these reports, the same dominant v-c groups were found in the Ticino in 1976 (18) and again in 1990 (32).

Sexual recombination of different v-c genotypes is the main source of v-c group diversity in *C. parasitica* (6). Information from several European countries (Table 1) suggests that perithecia, the sexual fruiting bodies of *C. parasitica,* are present, although they are not very frequent in most areas. The presence of perithecia, however, does not necessarily indicate that recombination has occurred, because the sexual stage can also be produced by self-fertilization (95, 110). If sexual reproduction occurs in a population, an increasing number of v-c groups can be expected, unless polymorphism at the v-c loci is very limited. This might be the case in Switzerland, where crosses showed that strains of the two dominant v-c groups differ only at a single v-c locus (D Rigling, unpublished data). Both low v-c polymorphism and limited sexual reproduction appear to have contributed to the low v-c group diversity in Europe.

FIELD APPLICATION OF HYPOVIRULENCE

The goal of large-scale application of hypovirulent strains of *C. parasitica* is the rapid and durable healing of the treated cankers. Inoculations of active cankers with hypovirulent strains healed the cankers when strains with the appropriate v-c group were used (18, 68). Mixtures of different hypovirulent strains often proved superior to a single strain (128). In addition, treatments of forest stands, where little hypovirulent inoculum is present, may promote dissemination of hypovirulent strains.

From 1967 to 1972 Grente & Berthelay-Sauret (66, 67) initiated field trials in 12 chestnut orchards in southern France. The incidence of disease was low: 1 to 10 disease foci/ha were present with 5 to 10 cankers each and hypovirulence was rare. On an area of 20 hectares 200 cankers were inoculated in the fall with one compatible hypovirulent strain. Most treated cankers did not expand until the next spring and none was lethal, compared to 5–8% of the untreated cankers. Four years later nontreated cankers also showed signs of healing. New healing cankers subsequently appeared and mortality decreased within a radius of 5 m from the treated cankers. Another trial failed because an incompatible hypovirulent strain was used. Several successful field applications followed in different regions of France.

These results were so promising that a biocontrol program, supported by the Ministry of Agriculture, was established in France to assist chestnut growers (67).

FRENCH PROTOCOL FOR FIELD APPLICATION After the v-c group of the specific *C. parasitica* is identified, an adjusted mixture of hypovirulent strains is produced, packed in tubes, and distributed to chestnut growers. The edge of the canker is defined by removing a thin layer of bark before applying the hypovirulent mixture into holes (2–3 cm apart) around the cankers (Figure 4; 67, 133). Mixtures of hypovirulent strains of *C. parasitica*, adjusted to the different chestnut-growing regions, are available commercially in France. Use is recommended for chestnut production in plantations.

A field test conducted by "CTGREF groupement de Grenoble" in 1975 on 4 hectares of forest stands (coppice and young plantings) (97) in the Massif les Maures, where hypovirulence was rare, was not encouraging. Although healing of treated cankers could be observed, new cankers developed and hypovirulence did not spread in the following five years. A further trial on 4 hectares of coppice in the Cevennes was also not very successful, with only 37% of the treated cankers healing. The main conclusion from these trials was that the persistence and dissemination of hypovirulent strains in forest stands is not reliable, especially when the forests are poorly managed. Blight control, however, is possible in well-maintained orchards, where all cankers must be treated with a selected mixture of hypovirulent strains (A Soutrenon, personal communication).

Field trials in Italy gave good results in areas where many active cankers were present and hypovirulence was rare. For example, in the Comunità Montana 'Valle Seriana' (Bergamo) only one hypovirulent isolate of *C. parasitica* was found and it was incompatible with the 42 resident virulent isolates (59). A total of 233 cankers (17 cankers/ha) in forest and coppice stands in this area were treated with a mixture of five hypovirulent strains that were compatible with the local virulent population. Application techniques similar to those in France were used. Within six years the treated cankers were all healing or healed. The frequency of untreated active cankers decreased from 41% to 25% in the treated plots compared to a 30% increase in the untreated control plots, indicating a natural dissemination of hypovirulence (29). Similar satisfactory results were obtained from other trials in Terni, Potremoli (80), and South Tyrol (93).

These encouraging results demonstrated that in areas where hypovirulence is low, field application of hypovirulent strains of *C. parasitica* promotes the dissemination of hypovirulence and lessens the severity of blight.

In 1993, a large trial was started in southern Italy on 3000-ha chestnut stands

(34). 30 cankers/ha were inoculated with a mixture of four hypovirulent strains with a wide conversion capacity. Treatment of 3000 seedlings is currently underway in South Tyrol, where hypovirulence is rare and its dissemination is restricted because of the isolation of chestnut stands (1).

Application of hypovirulent strains will be especially useful in regions at the front of the epidemic of chestnut blight, where hypovirulence is not present and may not invade naturally because the chestnut stands with hypovirulence are too far away.

ORCHARD PRACTICE AND SILVICULTURAL MANAGEMENT In chestnut-producing orchards where branch mortality reduces nut yield, sanitation practices include regular pruning of cankered branches and the subsequent treatment of the wounds with fungicides. As *C. parasitica* sporulates profusely on dead wood, removal of diseased branches and trees in combination with a shorter rotation period should reduce chestnut blight in orchards and high forests (90). However, since dead wood is also colonized with white *C. parasitica* (139), it may also serve as a source of hypovirulence. To promote the dissemination of hypovirulence it is proposed to leave stems with healing cankers in forest stands (124).

GRAFTING Nut-producing varieties of *C. sativa* are propagated by various grafting techniques, such as crown grafts on young plants or with whip and tongue grafts on 1-year old seedlings. Graft unions are especially susceptible to attack by *C. parasitica*. There is a substantial difference in susceptibility between chestnut varieties (62). Although blight attacks are negligible in nurseries, the loss may be considerable in orchards and woods (124). The antagonistic effect of soil compressions protected graft unions from blight attack (105). A commercially available tree wax based on *Bacillus subtilis* (T Turchetti, personal communication), was useful in protecting grafts in chestnut forests (123).

CONCLUDING REMARKS

At the beginning of the epidemic of chestnut blight *C. parasitica* devastated many chestnut stands in Europe. Meanwhile, however, hypovirulence developed naturally or after field release. Only stands at the front of the epidemic, where hypovirulence has not yet appeared, are still threatened by blight. Where hypovirulence arose and how it spreads has not been fully explained.

Unfortunately, this favorable development has not, with a few exceptions (7, 57) occurred in North American chestnut stands. The reason may lie in the host, the pathogen, and the viral dsRNA population. Compared to *C. dentata,*

C. sativa is less susceptible to blight. Slower canker development and delayed mortality may allow more time for hypovirulent strains to infect and subsequently convert cankers. The high v-c group diversity of populations of *C. parasitica* in North America compared to Europe may be another reason why the dissemination of hypovirulence has been unsuccessful in America. Finally, the viral dsRNA that causes hypovirulence in Europe is different from most naturally occurring dsRNAs detected in American isolates of *C. parasitica*. Attempts to improve hypovirulence by genetic manipulation of both the virus and the pathogen are underway (103) and await field performance tests.

The phenomenon of hypovirulence could serve as a model strategy for self-sustaining biocontrol of plant diseases. Similar control strategies could be applied to other diseases (104).

Chemical control of pathogens in forest trees is often not feasible and poses risks to the environment, and resistance breeding is incompatible with the aim of preserving a genetically broad tree population. However, since hypovirulence is self-sustainable (potentially after a single application), it is of special interest in controlling diseases of long-lived plants such as forest trees.

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Literature Cited

1. Aichner A, Minerbi S, Pernstich H, Schwarzer S. 1992. Kastanienrindenkrebs in Südtirol: Biologische Bekämpfungsmassnahmen. In *Wie Gesund Sind Unsere Wälder?*, ed. Forstwirtschaftsinspektorat Bozen, 1992:35–43. Autonome Provinz Bozen Südtirol
2. Allegri E. 1966. Risultati sulla sperimentazione del castagno giapponese e del castagno cinese e loro possibilità di impiego in Italia. In *Atti Convegno Int. sul Castagno*, ed. BS Dalmazzo, pp. 299–306. Ist. Graf. Bertello. Uff. Stampa Camera Comm. Ind. Artig. Agric. Cuneo
3. Alvisi F. 1993. Economic and commercial aspects of chestnut-growing. *Int. Congr. Chestnut* Spoleto:24 (Abstr.)
4. Anagnostakis SL. 1981. Stability of double-stranded RNA components of *Endothia parasitica* through transfer and subculture. *Exp. Mycol.* 5:236–42
5. Anagnostakis SL. 1987. Chestnut blight: the classical problem of an introduced pathogen. *Mycologia* 79:23–37
6. Anagnostakis SL. 1988. *Cryphonectria parasitica*, cause of chestnut blight. *Adv. Plant Pathol.* 6:123–36
7. Anagnostakis S. 1990. Improved chestnut tree condition maintained in two Connecticut plots after treatments with hypovirulent strains of the chestnut blight fungus. *For. Sci.* 36:113–24
8. Anagnostakis SL. 1992. Diversity within populations of fungal pathogens on perennial parts of perennial plants. In *The Fungal Communities*, ed. GC Carrol, DT Wicklow, pp. 183–92. New York: Dekker. 2nd ed.
9. Anagnostakis SL, Aylor DE. 1984. The

effect of temperature on growth of *Endothia (Cryphonectria) parasitica* in vitro and in vivo. *Mycologia* 76:387–97
10. Anagnostakis SL, Day PR. 1979. Hypovirulence conversion in *Endothia parasitica*. *Phytopathology* 69:1226–29
11. Anagnostakis SL, Hau B, Kranz J. 1986. Diversity of vegetative compatibility groups of *Cryphonectria parasitica* in Connecticut and Europe. *Plant Dis.* 70: 536–38
12. Anagnostakis SL, Kranz J. 1987. Population dynamics of *Cryphonectria parasitica* in a mixed-hardwood forest in Connecticut. *Phytopathology* 77:751–54
13. Anagnostakis SL, Waggoner PE. 1981. Hypovirulence, vegetative incompatibility, and the growth of cankers of chestnut blight. *Phytopathology* 71:1198–202
14. Arrigoni A. 1950. La malattia dell'inchiostro ed il cancro della corteccia del castagno. *Agric. Ticin.* 40–42:1–8
15. Baldacci E, Picco D. 1947. Il cancro del castagno. *Humus* 3:6–8
16. Bazzigher G. 1964. Die Ausbreitung der *Endothia*-Seuche im Kanton Tessin. *Schweiz. Z. Forstwes.* 115:320–30
17. Bazzigher G. 1981. Selection of blight-resistant chestnut trees in Switzerland. *Eur. J. For. Pathol.* 11:199–207
18. Bazzigher G, Kanzler E, Kübler T. 1981. Irreversible Pathogenitätsverminderung bei *Endothia parasitica* durch übertragbare Hypovirulenz. *Eur. J. For. Pathol.* 11:358–69
19. Bennett AR, Hindal DF. 1989. Mycelial growth and oxalate production by five strains of *Cryphonectria parasitica* in selected liquid culture media. *Mycologia* 81:554–60
20. Biraghi A. 1946. Il cancro del castagno causato da *Endothia parasitica*. *Ital. Agric.* 7:1–9
21. Biraghi A. 1947. Una gravissima minaccia per i nostri castagneti: il "cancro della corteccia". *Ital. For. Mont.* II/1:1–9
22. Biraghi A. 1948. Prove di inoculazione di castagni con *Endothia* sp. isolata in Biscaglia. *Ann. Sper. Agric.* 2:687–91
23. Biraghi A. 1949. Sulla lotta contro le malattie del castagno. Il problema fitopatologico. *Ital. For. Mont.* 4:64–73
24. Biraghi A. 1953. Possible active resistance to *Endothia parasitica* in *Castanea sativa*. *Rep. Congr. Int. Union For. Res. Org., 11th.* Rome
25. Biraghi A. 1954. Ulteriori notizie sulla resistenza di *Castanea sativa* Mill. nei confronti di *Endothia parasitica* (Murr.) And. *Boll. Staz. Patol. Veg.* 9:149–57
26. Biraghi A. 1955. Il "cancro della corteccia" ed i suoi riflessi sulla crisi del castagno. *Ital. For. Mont.* 10:1–11
27. Biraghi A. 1960. L'attuale situazione del "cancro della corteccia" del castagno in Italia. *Schweiz. Z. Forstwes.* 7:341–47
28. Bisiach M, De Martino A, Gobbi E, Intropido M, Vegetti G. 1988. Studies on chestnut blight: activity report. *Riv. Patol. Vegetal. S. IV,* 24:3–13
29. Bisiach M, De Martino A, Intropido M. 1991. Nuove esperienze di protezione biologica contro il cancro della corteccia del castagno. *Frutticoltura* 12:55–58
30. Bissegger M. 1993. *Populationsstruktur und -dynamik von Cryphonectria (Endothia) parasitica in Schweizer Edelkastanienwäldern (Castanea sativa).* PhD thesis. ETH Zürich. 142 pp.
31. Bissegger M, Heiniger U. 1991. Chestnut blight (*Cryphonectria parasitica*) north of the Swiss Alps. *Eur. J. For. Pathol.* 21:250–52
32. Bissegger M, Heiniger U. 1992. Population structure of *Cryphonectria parasitica* in Swiss chestnut stands. In *Proc. Int. Chestnut Conf.*, pp. 143–47. Morgantown, WV.
33. Bissegger M, Sieber TN. 1994. Assemblages of endophytic fungi in young, healthy coppice shoots of *Castanea sativa*. *Mycologia*. In press
34. Calza CA. 1993. Biological control of chestnut blight: large-scale application techniques. *Int. Chestnut Conf.*, Spoleto. In press
35. Carpenter CE, Mueller RJ, Kazmierczak P, Zhang L, Villalon DK, Van Alfen NK. 1992. Effect of a virus on accumulation of a tissue-specific cell-surface protein of the fungus *Cryphonectria (Endothia) parasitica*. *Mol. Plant-Microbe Interact.* 4:55–61
36. Chen B, Choi GH, Nuss DL. 1993. Mitotic stability and nuclear inheritance of integrated viral cDNA in engineered hypovirulent strains of the chestnut blight fungus. *EMBO J.* 12:2991–98
37. Choi GH, Larson TG, Nuss DL. 1992. Molecular analysis of the laccase gene from the chestnut blight fungus and selective suppression of its expression in an isogenic hypovirulent strain. *Mol. Plant-Microbe Interact.* 5:119–28
38. Choi GH, Nuss DL. 1992. Hypovirulence of chestnut blight fungus conferred by an infectious viral cDNA. *Science* 257:800–3
39. Choi GH, Nuss DL. 1992. A viral gene confers hypovirulence-associated traits to the chestnut blight fungus. *EMBO J.* 11:473–77
40. Choi GH, Pawlik DM, Nuss DL. 1991. The autocatalytic protease p29 encoded

by a hypovirulence-associated virus. of the chestnut blight fungus resembles the potyvirus-encoced protease HC-pro. *Virology* 183:747–52

41. Choi GH, Shapira R, Nuss DL. 1991. Cotranslational autoproteolysis involved in gene expression from a double-stranded RNA genetic element associated with hypovirulence of the chestnut blight fungus. *Proc. Natl. Acad. Sci. USA* 88:1167–71
42. Conedera M. 1991. La situazione del cancro corticale del castagno (*Cryphonectria (Endothia) parasitica* [Murr.] Barr.) al sud delle alpi (Svizzera meridionale). *Schweiz. Z. Forstwes.* 142:283–98
43. Darpoux H, Ridé M, Bondoux P. 1957. Apparition de foyers d'"*Endothia parasitica*" sur châtaigniers en France. *CR Acad. Agric. France* 43:670–74
44. Day PR, Dodds JA, Elliston JE, Jaynes RA, Anagnostakis SL. 1977. Double-stranded RNA in *Endothia parasitica*. *Phytopathology* 67:1393–96
45. Delen N. 1975. Distribution and the biology of chestnut blight [*Endothia parasitica* (Murrill) Anderson and Anderson]. *J. Turk. Phytopathol.* 4:93–113
46. Delen N. 1980. Studies on the control possibilities of chestnut blight [*Endothia parasitica* (Murr.) A. and A.] in Turkey. *J. Turk. Phytopathol.* 9:27–47
47. Del Guerra L. 1948. Le malattie parassitarie ed i castagneti del Piemonte e della Liguria. *Ital. For. Mont.* 3:266–87
48. Del Guerra L. 1948. Le mallatie parassitarie ed i castagneti del Piemonte e della Ligura. *Ital. For. Mont.* 3:352–75
49. Diamandis S. 1989. Chestnut blight on the peninsula of M. Athos. *Sci. Ann.* LB/1:317–28
50. Elliston JE. 1978. Pathogenicity and sporulation of normal and diseased strains of *Endothia parasitica* in American chestnut. *Proc. Am. Chestnut Symp.* Morgantown:95–100
51. Elliston JE. 1982. Effects of selected North American and Italian cytoplasmic hypovirulence agents on North American and Italian strains of *Endothia parasitica*. Proc. USDA For. Service Am. Chestnut Coop. Meet., Morgantown:134–40
52. Elliston JE. 1982. Hypovirulence. *Adv. Plant Pathol.* 1:1–33
53. Fahima T, Kazmierczak P, Hansen DR, Pfeiffer P, Van Alfen NK. 1993. Membrane-associated replication of an unencapsidated double-strand RNA of the fungus, *Cryphonectria parasitica*. *Virology* 195:81–89
54. Fenaroli L. 1945. *Il Castagno*, Vol. I. Roma: Ramo Edit. Agric. 222 pp.
55. Figl KH, Donaubauer E. 1991. Untersuchungen über den Erreger des Kastanienrindenkrebses *Cryphonectria parasitica*, sowie Möglichkeiten der biologischen Bekämpfung durch hypovirulente Stämme. Forstschutz aktuell, Forstl. Bundesversuchsanst. *Wien* 7:1–3
56. Frigimelica G, Causin R, Mutto AS. 1993. Vegetative compatibility among isolates of an Italian population of *Cryphonectria parasitica*. *Int. Congr. Chestnut* Spoleto, p. 76 (Abstr.)
57. Fulbright DW, Paul CP, Garrod SW. 1988. Hypovirulence: a natural control of chestnut blight. In *Biocontrol of Plant Diseases*, ed. KG Muerji, KL Grey, 2:121–39. Boca Raton, FL: CRC Press
58. Garbelotto M, Frigimelica G, Mutto-Accordi S. 1992. Vegetative compatibility and conversion to hypovirulence among isolates of *Cryphonectria parasitica* from northern Italy. *Eur. J. For. Pathol.* 22:337–48
59. Gobbi E, Intropido M. 1984. Esperienze di protezione biologica del castagno dal cancro corticale. *1° convegno nazionale "Problemi fitopatologici delle pianti forestali" Venezia-Mestre*, pp. 93–96
60. Gobbi E, Locci R. 1989. Aspetti micologici e fitopatologici di *Cryphonectria parasitica*. *Micol. Ital.* 18:31–36
61. Gobbi E, Wang Y, Martin RM, Powell WA, Van Alfen NK. 1990. Mitochondrial DNA of *Cryphonectria parasitica*: Lack of migration between vegetatively compatible strains. *Mol. Plant-Microbe Interact.* 3:66–71
62. Govi G, Zechini d'Aulerio A. 1982. Resistenza varietale del castagno all'*Endothia parasitica*. *Inf. Fitopatol.* 9–10:59–63
63. Granata G, Sidoti A, Gullotto A, Pennisi AM. 1992. Incidenza del cancro della corteccia del castagno in Sicilia e prove di compatibilità vegetativa. *Tecn. Agric.* 1:1–10
64. Grandi C. 1958. Il cancro corticale del castagno ed il risanamento della zona pedemontana nel Cantone Ticino. *Schweiz. Z. Forstwes.* 109:375–91
65. Grente MJ. 1965. Les formes hypovirulentes d'*Endothia parasitica* et les espoirs de lutte contre le chancre du châtaignier. *Acad. Agric. France*:1033–36
66. Grente J. 1981. *Les variants hypovirulents de l'Endothia parasitica et la lutte biologique contre le chancre du*

châtaignier. PhD. dissertation. Lab. Biol. Vég., Univ. Bretagne Occid. 195 pp.
67. Grente J, Berthelay-Sauret S. 1978. Biological control of chestnut blight in France. *Proc. Am. Chestnut Symp.* Morgantown, WV. Jan. 4–5:30–34
68. Grente MJ, Sauret S. 1969. Pathologie végétale. —L'hypovirulence exclusive, phénomène original en pathologie végétale. *CR Acad. Sci.* 268:2347–50
69. Griffin GJ. 1986. Chestnut blight and its control. *Hortic. Rev.* 8:291–336
70. Halambek M. 1986. Chestnut blight in Yugoslavia. *Bull. OEPP/EPPO* 16:533–35
71. Halambek M. 1991. Hipovirulentni sojevi *Cryphonectria parasitica*-nova mogucnost za oporavak pitomog kestena. *Radovi* 26:223–40
72. Hansen DR, Van Alfen NK, Gillies K, Powell WA. 1985. Naked dsRNA associated with hypovirulence of *Endothia parasitica* is packaged in fungal vesicles. *J. Gen. Virol.* 66:2605–14
73. Havir EA, Anagnostakis SL. 1983. Oxalate production by virulent but not by hypovirulent strains of *Endothia parasitica*. *Physiol. Plant Pathol.* 23: 369–76
74. Hebard FV, Griffin GJ, Elkins JR. 1984. Developmental histopathology of cankers incited by hypovirulent and virulent isolates of *Endothia parasitica* on susceptible and resistant chestnut trees. *Phytopathology* 74:140–49
75. Heiniger U, Bissegger M. 1993. Population dynamics of *Cryphonectria parasitica*. *Int. Chestnut Conf.* Spoleto. In press
76. Heiniger U, Conedera M. 1992. Chestnut forests and chestnut cultivation in Switzerland. In *Proc. US For. Service, Am. Chestnut Coop. Meet.*, Morgantown, WV
77. Heiniger U, Stadler B. 1990. Kastanienrindenkrebs auf der Alpennordseite. *Schweiz. Z. Forstwes.* 141: 383–88
78. Hillman BI, Tian Y, Bedker PJ, Brown MP. 1992. A North American hypovirulent isolate of the chestnut blight fungus with European isolate-related dsRNA. *J. Gen. Virol.* 73:681–86
79. Huntley B, Birks HJB. 1983. *An Atlas of Past and Present Pollen Maps for Europe: 0–13,000 Years Ago*. Cambridge: Cambridge Univ. Press. 667 pp.
80. Intropido M, De Martino A, Bisiach M. 1987. Lotta biologica contro il cancro della corteccia del castagno. *Monti Boschi* 38:31–37
81. Intropido M, De Martino A, Pilastro V, Bisiach M. 1993. Vegetative compatibility and conversion of *Cryphonectria parasitica* isolates from different Italian regions. *Int. Chestnut Conf.* Spoleto. In press
82. Jaynes RA, De Palma NK. 1984. Natural infection of nuts of *Castanea dentata* by *Endothia parasitica*. *Phytopathology* 74:296–99
83. Juhàsovà G, Kamenickà A, Berthelay-Sauret S. 1993. Biological protection and regeneration of *Castanea sativa* Mill. by means of in vitro methods in Slovakia. *Int. Congr. Chestnut, Spoleto*, p. 78 (Abstr.)
84. Koonin E, Choi GH, Nuss DL, Shapira R, Carrington JC. 1991. Evidence for common ancestry of a chestnut blight hypovirulence-associated double-stranded RNA and a group of positive-strand RNA plant viruses. *Proc. Natl. Acad. Sci. USA* 88:10647–51
85. Kristic M. 1950. *Endothia parasitica* u nosoj zemlji. *Zastita Bilja Br.* 2:113–16
86. Kuhlman EG, Bhattacharyya H, Nash BL, Double ML, Mac Donald WL. 1984. Identifying hypovirulent isolates of *Cryphonectria parasitica* with broad conversion capacity. *Phytopathology* 74:676–82
87. Larson TG, Choi GH, Nuss DL. 1992. Regulatory pathways governing modulation of fungal gene expression by a virulence-attenuating mycovirus. *EMBO J.* 11:4539–48
88. L'Hostis B, Hiremath ST, Rhoads RE, Ghabrial SA. 1985. Lack of sequence homology between double-stranded RNA from European and American hypovirulent strains of *Endothia parasitica*. *J. Gen. Virol.* 66:351–55
89. Liang P, Chen K, Zhou S, Quan Y, Liu H. 1992. Isolation and conversion of dsRNA from hypovirulent strains of *Endothia parasitica* in China. *Acta Microbiol. Sin.* 32:253–61
90. Lopinto M. 1984. Efficacia della dendrochirurgia nella lotta contro il cancro corticale del castagno (*Castanea sativa* Mill.). In *Problemi fitopatologici delle piante forestali*, pp. 121–29. Venezia-Mestre. 1 convegno nazionale
91. Luisi N, Vannini A. 1985. Ipovirulenza in *Endothia parasitica*: sua caratterizzazione e contenimento naturale della malattia nei castagneti del viterbese. *Difesa delle piante* 2:197–206
92. MacDonald WL, Fulbright DW. 1991. Biological control of chestnut blight: use and limitations of transmissible hypovirulence. *Plant Dis.* 75:656–61
93. Maresi G, Minerbi S, Sottovia A,

Turchetti T. 1993. Der Kastanienrindenkrebs in Südtirol. *AFZ* 3:140–44
94. Martin RM, Van Alfen NK. 1991. The movement of viral-like RNA between colonies of *Cryphonectria parasitica*. *Mol. Plant-Microbe Interact.* 4:507–11
95. Milgroom MG, Lipari SE, Ennos RA, Liu Y-C. 1993. Estimation of the outcrossing rate in the chestnut blight fungus, *Cryphonectria parasitica*. *Heredity* 70:385–92
96. Milgroom MG, Mac Donald WL, Double ML. 1991. Spatial pattern analysis of vegetative compatibility groups in the chestnut blight fungus, *Cryphonectria parasitica*. *Can. J. Bot.* 69:1407–13
97. Ministere de l'agriculture. 1980. *Compte-rendu des observations faites par le C.T.G.R.E.F. sur des traitements de lutte biologique contre le chancre du ch!taignier* Endothia parasitica *dans le massif forestier des Maures*. Min. Agric. Cen. Tech. Génie Rural Eaux For., Group. Grenoble, Div. Prot. Nature. 75 pp.
98. Mlnkemyer. 1992. *Die Gefährdung der Kastanie auf der Iberischen Halbinsel durch Cryphonectria parasitica (Murr.) Barr mit Versuchen zur Hypovirulenz des Rindenkrebserregers*. Diploma thesis. Inst. Waldbau, Göttingen. 158 pp.
99. Mutto S, Del Sole E. 1985. Impostazione di un modello matematico dello sviluppo di *Endothia parasitica* nel territoria dei Colli Euganei. *Inf. Fitopatol.* 4:51–54
100. Nannelli R, Tuchetti T. 1989. Osservazioni preliminari sull'associazione di alcune specie di acari corticicoli con *Cryphonectria parasitica* (Murr.) Barr. (Preliminary observations on the association of some species of corticolous acari with *Cryphonectria parasitica* (Murr.) Barr.) *Redia* 72:581–93
101. Newhouse JR. 1990. Chestnut blight. A fungus that has ravaged the American chestnut is itself coming under biological attack. Plant pathologists hope the new parasite will eventually control the old one. *Sci. Am.* 7:74–79
102. Newhouse JR, Mac Donald WL, Hoch HC. 1990. Virus-like particles in hyphae and conidia of European hypovirulent (dsRNA-containing) strains of *Cryphonectria parasitica*. *Can. J. Bot.* 68: 90–101
103. Nuss DL. 1992. Biological control of chestnut blight: an example of virus-mediated attenuation of fungal pathogenesis. *Microbiol. Rev.* 56:561–76
104. Nuss DL, Koltin Y. 1990. Significance of dsRNA genetic elements in plant pathogenic fungi. *Annu. Rev. Phytopathol.* 28:37–58
105. Paci M, Tani A. 1982. Ulteriori risultati di esperienze sul castagno in Chianti. *Ital. For. Mont.* 37:188–202
106. Pavari A. 1947. La lotta contro un nuovo flagello dei nostri castagneti (*Endothia parasitica*). In *Atti della Accademia dei Georgofili*, pp. 1–19.
107. Pennisi AM, Magnano di San Lio G, Grasso S. 1992. Compatibilità vegetativa di isolati di *Cryphonectria parasitica* (Murr.) Barr ottenuti da castagneti in Calabria. *Petria* 2:1–10
108. Powell WA, Van Alfen NK. 1987. Differential accumulation of poly(A)+RNA between virulent and double-stranded RNA-induced hypovirulent strains of *Cryphonectria (Endothia) parasitica*. *Mol. Cell. Biol.* 7:3688–93
109. Powell WA, Van Alfen NK. 1987. Two nonhomologous viruses of *Cryphonectria (Endothia) parasitica* reduce accumulation of specific virulence-associated polypeptides. *J. Bacteriol.* 169:5324–26
110. Puhalla JE, Anagnostakis SL. 1971. Genetics and nutritional requirements of *Endothia parasitica*. *Phytopathology* 61:169–73
111. Rigling DH. 1989. *Hypovirulenz, dsRNA und Laccase-Aktivität bei Cryphonectria (Endothia) parasitica, dem Erreger des Kastanienrindenkrebses*. PhD Dissertation. Univ. Zürich. 82 pp.
112. Rigling D, Heiniger U, Hohl HR. 1989. Reduction of laccase activity in dsRNA-containing hypovirulent strains of *Cryphonectria (Endothia) parasitica*. *Phytopathology* 79:219–23
113. Rigling D, Van Alfen NK. 1991. Regulation of laccase biosynthesis in plant-pathogenic fungus *Cryphonectria parasitica* by double-stranded RNA. *J. Bacteriol.* 173:8000–3
114. Rigling D, Van Alfen NK. 1993. Extra- and intracellular laccase of the plant pathogenic fungus *Cryphonectria parasitica*. *Appl. Enviro. Microbiol.* 59: 3634–39
115. Russin JS, Shain L. 1985. Disseminative fitness of *Endothia parasitica* containing different agents for cytoplasmic hypovirulence. *Can. J. Bot.* 63:54–57
116. Russin JS, Shain L, Nordin GL. 1984. Insects as carriers of virulent and cytoplasmic hypovirulent isolates of the chestnut blight fungus. *J. Econ. Entomol.* 77:838–46
117. Schülepp H. 1961. Erhebungen über das Kastaniensterben im Kanton Tessin. *Mitt. EAFV* 37:159–69
118. Seemann D, Unger J-G. 1993. Rin-

denkrebs der Esskastanie in der Bundesrepublik Deutschland. *Nachrichtensbl. Dtsch. Pflanzenschutzdienst* 45: 120–22

119. Shain L. 1982. Strategies for enhancing dissemination of hypovirulence in *Endothia parasitica*: state of the art. In *Proc. US For. Serv., Am. Chestnut Coop. Meet.*, ed. HC Smith, WC Mac Donald, pp. 175–83. Morgantown
120. Shapira R, Choi GH, Hillman BI, Nuss DL. 1991. The contribution of defective RNAs to the complexity of viral-encoded double-stranded RNA populations present in hypovirulent strains of the chestnut blight fungus *Cryphonectria parasitica*. *EMBO J.* 10:741–46
121. Shapira R, Choi GH, Nuss DL. 1991. Virus-like gnenetic organization and expression strategy for a double-stranded RNA genetic element associated with biological control of chestnut blight. *EMBO J.* 10:731–39
122. Smith IM, McNamara DG, Scott PR, Harris KM. 1992. *Cryphonectria parasitica*. In *Quarantine Pests for Europe. Data Sheets on Quarantine Pests for the European Communities and for the European and Mediterranean Plant Protection Organization*, ed. IM Smith, DG McNamara, PR Scott, KM Harris, pp. 467–71. Cambridge: Univ. Press
123. Tomasi G, Turchetti T, Maresi G. 1989. Alcune esperienze sulla ricostituzione dei castagneti da frutto nella provincia di Trento. *Esper. Ric. (n.s.)* 19:61–72
124. Turchetti T. 1982. Hypovirulence in chestnut blight (*Endothia parasitica* [Murr.] And.) and some practical aspects in Italy. *Eur. J. For. Pathol.* 12:414–17
125. Turchetti T. 1984. Aspetti fitopatologici negli innesti di castagno. *I convegno nazionale. I problemi fitopatologici delle piante forestali. Venezia-Mestre*, pp. 97–106
126. Turchetti T. 1987. Prospettive di lotta biologica in patologia forestale. In *Convegno sulle avversità del bosco e delle specie arboree da legno*, pp. 265–76. Firenze
127. Turchetti T, Maresi G. 1988. Mixed inoculum for the biological control of chestnut blight. *Bull. OEPP* 18:67–72
128. Turchetti T, Maresi G. 1991. Innoculation trials with hypovirulent strains of *Cryphonectria parasitica*. *Eur. J. For. Pathol.* 21:65–70
129. Turchetti T, Sottovia A, Maresi G, Minerbi S. 1992. Il cancro della corteccia del castagno in Alto Adige. *Inf. Fitopatol.* 4:45–48
130. Van Alfen NK. 1982. Biology and potential for disease control of hypovirulence of *Endothia parasitica*. *Annu. Rev. Phytopathol.* 20:349–62
131. Van Alfen NK, Jaynes RA, Anagnostakis SL, Day PR. 1975. Chestnut blight: biological control by transmissible hypovirulence in *Endothia parasitica*. *Science* 189:890–91
132. Varley DA, Podila GK, Hiremath ST. 1992. Cutinase in *Cryphonectria parasitica*,the chestnut blight fungus: suppression of cutinase gene expression in isogenic hypovirulent strains containing double-stranded RNAs. *Mol. Cell. Biol.* 12:4539–44
133. Vrot F, Grente J. 1985. Le chancre de l'écorce du châtaignier. *Phytoma-Déf. Cult.* Mars:35–37
134. Xenopolous S. 1982. Severity of chestnut blight disease and the pathogenicity of the causal fungus *Endothia parasitica* in Greece. *Eur. J. For. Pathol.* 12:316–26
135. Xenopoulos S. 1981. Degree of pathogenicity and test for vegetative incompatibility of normal and diseased strains of *Endothia parasitica*. *Dasiki Ereyna* 2:469–82
136. Xenopoulus SG. 1988. Pathogenicity studies of several virulent and cytoplasmic hypovirulent strains of *Endothia parasitica* in European chestnut. *Dasiki Ereyna* 2:91–101
137. Zambonelli A, Zechini d'Aulerio A. 1986. Combatibilità vegetativa e sessuale di ceppi di *Endothia parasitica* (Murr.) and isolati in un medesimo castagneto. *Inf. Fitopatolog.* 10:39–42
138. Zambonelli A, Zechini d'Aulerio A. 1987. Aspetti biologici e genetici di *Endothia parasitica* in coltura. *Micol. Ital.* 3:165–72
139. Zechini d'Aulerio A, Carbone W, Della Valle E. 1982. Il cancro della corteccia del castagno: prove di lotta biologica. *Inf. Fitopatolog.* 6:43–46
140. Zechini d'Aulerio A, Zambonelli A. 1987. Virulenza ed ipovirulenza di *Endothia parasitica* in un castagneto da frutto. *Monti Boschi* 2:20–24
141. Zhang L, Kim D-H, Churchill A, Sun YB, Razmierczak P, Baasiri R, Van Alfen NK. 1993. Expression of putative mating type a pheromone genes of *Cryphonectria parasitica* are repressed by a fungal virus. In *Fungal Genetics Conference Asilomar, 17th* p. 67 (Abstr.). Pacific Grove, CA
142. Zoina A. 1987. Il cancro della corteccia: conoscenze e prospettive. *Agric. Camp.* 10(Suppl.):24–40