STRATEGIES FOR ENHANCING DISSEMINATION OF HYPOVIRULENCE IN *ENDOTHIA PARASITICA:* STATE OF THE ART

L. Shain

Department of Plant Pathology
University of Kentucky, Lexington, KY 40546

ABSTRACT. --The chestnut blight epidemics of eastern United States and Italy were compared. In spite of substantial differences in their ecosystems (host species, forest community, rainfall distribution, major soil types), the epidemics caused by virulent (V) strains of Endothia parasitica proceeded apparently at similar rates, i.e. ca. 18 and 23 miles per year for eastern United States and Italy, respectively. This was facilitated by the abundant production of conidia, which are spread by a variety of agents, and wind-blown ascospores. Healing cankers attributed to hypovirulent (H) strains of the pathogen were observed in Italian chestnut forests 12 to 15 years after attack by V strains. Within 20 to 30 years, H strains succeeded in checking blight in Italy. The rate of spread of H strains in Italy, therefore, was about the same as that for V strains. In the absence of significant spread of H strains in the eastern United States during the past 80 years, it seems unlikely that such spread will occur naturally in the foreseeable future. It may, however, be possible to establish disseminating H strains by knowledgeable intervention. Perhaps the best hope for enhancing dissemination of H strains lies in an understanding of how hypovirulence spreads where it is spreading. Intensive studies in these areas (i.e. Italy, France, and perhaps most significantly, western Michigan) should seek to determine rates and patterns of spread, rates of increase of H strains, host density, incidence of vectors or other novel relationships affecting dissemination, contribution of asexual and sexual sporulation, and inoculum density of H strains. Slow rates of local spread need not preclude success as H strains can be established artificially in many places throughout the natural range of American chestnut. Chestnut blight continues unabated, with rare possible exceptions, in the eastern United States while the epidemic in southern Europe has subsided. This has provided hope that chestnut blight can be controlled eventually in the United States. An understanding of how hypovirulent (H) strains of Endothia parasitica were disseminated in Europe may bear heavily on the outcome of efforts to combat blight in North America. To address this question, it seems appropriate to first compare the epidemics of North America and Europe with particular emphasis on factors which could have influenced the dissemination of virulent (V) and H strains. This information could be instructive as we explore possible strategies to enhance and detect dissemination of H strains within the natural range of American chestnut.

The Chestnut Blight Epidemics of Eastern United States and Italy

Most of the documented information about chestnut blight in Europe deals with the Italian epidemic. A comparison therefore will be made between the epidemics in eastern United States and Italy (Table 1). I confess an uneasiness about presenting the blight situation in Italy as I have not observed it personally. My interpretation of what has occurred is based solely upon a relatively sparse literature and personal communication. The source of uneasiness therefore lies in the danger of my misinterpretation of the printed word or my failure to have asked the right questions.

Table 1. Characteristics of chestnut - growing areas and of chestnut - blight epidemics in the eastern United States and Italy

	Eastern United States	Italy
HOST AND SITE		
Major host	*Castanea dentata* (Marsh.) Borkh.	*Castanea sativa* Mill.
Range	1200 x 500 = 600,000 miles^2	700 x 90 = 63,000 miles^2
Latitude	N 34^o - 45^o	N 38^o - 46^o
Major soil types	Gray-brown, red-yellow, podzolic soils	Calcimorphic, brown forest soils
Rainfall distribution	Throughout year	Summer drought
Prevailing wind direction	Southwest	Northwest
Forest community	Mixed hardwoods	Frequently pure, dense stands of selected cultivars
CHESTNUT BLIGHT EPIDEMIC		
First report of blight	1904	1938
Years to spread throughout range	ca. 40 years	ca. 30 years
Predominant direction of spread	Southwest	Variable
Rate of spread in predominant direction	900 miles ÷ 40 years = 23 miles/year	550 miles ÷ 30 years = 18 miles/year
Spread of hypovirulence through range	Negligible in 80 years	20 to 30 years

The chestnut blight epidemics of Italy and the eastern United States are separated by about 5,000 miles. It is not surprising, therefore, that these epidemics differ in major host species, associated plant and animal communities, and certain features of physical environment. Some of these differences will be discussed briefly with particular emphasis on how they may have effected dissemination of V and H strains of *E. parasitica.*

Dissemination of V Strains

While both the European and American chestnuts are highly susceptible to V strains, it is possible that the former is slightly less susceptible than the latter (Graves 1950). Most infection probably occurs during the growing season (Anderson 1913a; Double this proceedings). During moist conditions, asexual conidia are extruded from pycnidia and sexual ascospores are forcibly ejected through a film of water covering the ostioles of perithecia (Anderson and Babcock 1913). The production and dissemination of these spores, therefore, would be favored more by the moist summers of the eastern United States than by the dry summers characteristic of the Mediterranean climate of Italy (Watson 1968). Nonetheless, the average rate of spread in the predominant direction of spread was quite similar for the two epidemics. Blight was present throughout the range of American chestnut within about 40 years after it was first reported in New York City in 1904 (Beattie and Diller 1954). Spread of 900 miles to the Southwest therefore occurred at an average rate of about 23 miles per year. In Italy, blight spread throughout chestnut growing regions in about 30 years after it was first observed in the vicinity of Genoa in 1938 (Mittempergher 1978). Blight in Italy therefore spread 550 miles to the Southeast at an average rate of about 18 miles per year.

The rate of disease increase in American chestnut was extremely rapid in some areas. For example, detailed observations were made in a small area within the advancing edge of infection in the vicinity of Bluemont, Virginia, during 1913 to 1914 (Rogers and Gravatt 1915). This community is about 20 miles east of Winchester and 250 miles southwest of New York City. Of the 140 chestnut trees in this area of about 1,300 m^2, 50 (29 percent)were infected in 1913 and 83 (59 percent)were infected in 1914. From this data a rate of disease increase (r) of 1.26 can be calculated (Vanderplank 1963). At this rate, disease would increase from 1 percent to 90 percent in 5 to 6 years. This is in general agreement with the rates of disease increase observed in study plots in Maryland and Virginia (Gravatt and Gill 1930). While the number of infected trees doubled in 1 year in the Bluemont study, it is interesting to note that the total area and number of cankers increased by factors of about 5 and 3, respectively, during the same time. This information provides an opportunity to relate canker area, which was ca. 19,300 cm^2 at the 1913 observation, with rate of disease increase. Merrill (1967) reported r values of 1.42 and 0.83 for the chestnut blight epidemics in Pennsylvania and Connecticut, respectively. I have not seen data that would permit the calculation of r values for the Italian epidemic.

Ascospores are wind disseminated after their forcible ejection from perithecia. It has been suggested that these spores played a major role in the spread of V strains (Anderson 1913b; Heald et al. 1915). It is curious, however, that spread in Italy appeared to be multidirectional (Baldacci and Orsenigo 1952) with a prevailing northwest wind, and the most rapid spread in the eastern United States was to the Southwest (Metcalf and Collins 1911; Gravatt and

Marshall 1926) into a prevailing southwest wind. It could be argued that spread was in the direction of the greatest concentration of susceptible hosts. But, the early spread of the wind-disseminated gypsy moth was to the North and Northeast, i.e. with the prevailing wind, after its introduction into Massachusetts in 1869 (Campbell 1979), even though the colder northern temperatures are sometimes lethal to eggs (Summers 1922) and the greater concentration of preferred hosts is to the South. While the significance of this is unclear, it may be a reflection of the more rapid growth of the pathogen and concomitant production of inoculum in the warmer South as suggested by Stevens (1917). Lack of correlation between prevailing wind direction and direction of spread also suggests that agents of dissemination in addition to wind-blown ascospores could have played a significant role in disease spread. Indeed, some additional agents have been implicated. Those mentioned most prominantly were insects, birds, and the movement of infected host material by man.

The major differences in geographic area, soil type (Watson 1968), and plant community dictate also major differences in the insect fauna associated with the epidemics of eastern United States and Italy. Representatives of major insect taxa associated with one epidemic, however, would also be expected to be associated with the other. Insects are the vectors of many plant pathogens (Harris and Maramorsch 1980; Leach 1940), and they received early attention as possible vectors of chestnut blight fungus. Although viable spores were collected from a variety of insects including ants and beetles (Anagnostakis this proceedings; Anderson and Babcock 1913; Studhalter and Ruggles 1915) and mites (Wendt et al. this proceedings), I am unaware of any work that clearly satisfies the rules of proof for insect transmission (Leach 1940). Consequently, opinion was mixed as Studhalter and Ruggles (1915) concluded that insects were important vectors whereas Craighead (1916) concluded otherwise because the insects that frequent blight cankers rarely visit healthy trees. Craighead suggested that the significance of insects in disease dissemination was in their providing suitable wounds for infection. While this may be true, it has not been established experimentally, Of the many types of wounds inoculated by Anderson and Babcock (1913), insect holes were among the very few that did not become infected. This could reflect an effect of wound condition or age. We need to know more about what constitutes an infectable wound, particularly as it may relate to the dissemination of H strains.

Conidia of *E, parasitica* were recovered from the bodies of six species of birds in Pennsylvania during the winter and spring of 1913 (Heald and Studhalter 1914). Most of these birds which included the brown creeper, *Certhia familaris;* downy woodpecker, *Dendrocopus pebescens;* golden-crowned kinglet, *Regulus satrapa satrapa;* Junco, *Junco hyemalis;* white-breasted nuthatch, *Sitta carolinensis;* and sapsucker, *Sphyrapicus varius;* were killed either during or shortly after visiting chestnut blight cankers. Birds shot within 2 to 4 days of significant rainfall carried the highest number of conidia (up to 757,000). The cedar waxwing, *Bombycilla cedrorum,* recently was added to the list of birds that has carried inoculum (Scharf and DePalma 1981). Circumstantial evidence therefore suggests that vectoring could have occurred by highly active mobile birds that visit both cankered and healthy trees. The sapsucker seems to be a particularly viable candidate in that it is migratory and it feeds on insects as well as sap obtained from wounds it inflicts into the xylem of healthy trees (Bent 1939). Some of the genera of birds that carried spores in the eastern United States occur also in Italy, e.g. creepers,

woodpeckers, kinglets, nuthatches, and waxwings (Bruun and Singer 1970). Small mammals have been implicated as possible vectors for local spread (Scharf and DePalma 1981).

Movement of infected host material by man probably provided the inoculum for some of the infection centers that occurred well in advance of the main epidemic (Anderson and Babcock 1913; Gravatt and Marshall 1926). There is little doubt that such movement enabled V strains to gain initial entry into the eastern United States and Italy. Differences in the culture of chestnut in the two areas suggest that movement of infected host material may have played a greater role in dissemination in Italy. The American chestnut occurred usually in natural stands of mixed hardwoods whereas chestnut frequently is grown in pure dense stands of selected cultivars in Italy (Mittempergher 1978). The intensive culture of chestnut in Italy, including movement of specific cultivars throughout the country, therefore offered considerable opportunity for the movement of infected material.

The rapid rate of spread of V strains in both the eastern United States and Italy demonstrates that efficient means for dissemination were not limiting.

Dissemination of H Strains

Healing cankers were first observed near Genoa, Italy, about 12 years after the first report of blight in the same area. Healing cankers soon were observed in other areas of older infection, i.e. about 15 years after initial blight (Biraghi 1953; Mittempergher 1978) and within 20 to 30 years H strains "succeeded in checking the disease in Italy" (Grente and Berthelay-Sauret 1978). This indicates that the rates of dissemination of V and H strains were similar in Italy. The rate of increase of H strains also was substantial. The percentage of H strains isolated from cankers in the Piedmont increased from about 25 percent in 1972 to about 75 percent in 1977 (Grente and Berthelay-Sauret 1978). From this an r value of 0.44 can be calculated. If this rate of increase were sustained, H strains would increase from 1 percent to 90 percent in 15 to 16 years. The rate of increase of H strains also was considerable following artificial inoculation of cankers with H strains in France. An r value in excess of 0.68 can be calculated if we assume from data provided by Grente and Berthelay-Sauret (1978) that a hectare was "completely healed" 10 years after 10 of its 100 cankers were treated with H strains. Radial spread of H strains around inoculation sites proceeded at 1 to 2 m per year. While this figure is not impressive, particularly to some (Kuhlman 1981), it may not be as bad as it seems because the area of spread is a function of the square of the radius. The area of spread therefore quadruples with each doubling of time after successful inoculation. For example, a radial spread of 2 m per year translates into an area of spread of 314 m^2, 1,257 m^2, and 5,027 m^2 after 5, 10, and 20 years, respectively. It might be mentioned that the annosus root-rot epidemic also spreads locally at a rate of about 1 m per year (Berry 1968). Few would argue that this disease spreads too slowly to be of consequence.

In an evaluation of possible causes for the gross difference in spread of H strains in Italy and the eastern United States, differences in major host species should not be ignored. It is conceivable that host responses to H strains by *C. sativa* are more favorable than those by *C. dentata* for the development of the type cankers which are most conducive for dissemination

(e.g. persistent, nonlethal) (Elliston 1981). The recent observations of Weidlich et al. (this proceedings) and Brewer (this proceedings), however, suggest that American chestnut is capable of supporting this type of H canker in Michigan.

It seems unlikely that windblown ascospores played a role in the dissemination of H strains in Italy. Few perithecia are produced by H strains (Elliston 1978; Turchetti 1978) and the dsRNA associated with hypovirulence has not been shown to be transmitted through the sexual stage (Anagnostakis personal communication). The role of conidia is less clear. Fewer pycnidia are produced by H strains than by V strains (Elliston 1978) but dsRNA sometimes is transmitted to conidia by the former (Anagnostakis; Elliston personal communication). The number of conidia containing dsRNA would dictate the potential of these spores for dissemination of H strains.

As with V strains, other agents that could have played a role in the dissemination of H strains include birds, small mammals, insects, and the movement of infected host material by man.

Even though some of the genera of birds that carried inoculum of V strains in the eastern United States occur also in Italy, as mentioned above, it seems unlikely that they played a significant role in the recent dissemination of H strains. During a 3-week tour in 1978, Elliston (personal communication) did not see or hear birds or small mammals in the chestnut forests he visited in Italy. Evidence of woodpecker feeding at cankers also was riot observed. Turchetti (personal communication) indicated to Elliston that the bird and squirrel populations of Italian forests have been hunted almost to extinction.

Prospects for insect dissemination of H strains seem better although information on insects associated with blight cankers in Italy is lacking. The serious reservations of Craighead (1916) with regard to dissemination of V strains are not as applicable to H strains. As pointed out earlier (Day 1978), insects which frequent blight cankers could facilitate movement of H inoculum to V cankers. The mention by Mittempergher (1978) that the pure chestnut stands are excellent grazing grounds suggests that they may be used extensively for this purpose in Italy. This could effect among other things, the number and species of insect residents. Ants were mentioned by Grente (Day 1978) as possible vectors of H strains in France. Studies on possible insect vectoring of H strains, including the use of surrogate fungal attractants, were reviewed earlier (Russin et al. this proceedings).

The extensive movement of host material by man in Italy could have played a more important role in the long-distance spread of H strains than V strains due to the limited sporulation of the former.

Prospects for the Dissemination of H Strains in the eastern United States

Hypovirulent strains spread through the Italian chestnut forest in 20 to 30 years (Grente and Berthelay-Sauret 1978) or about as quickly as V strains. In the absence of significant spread of H strains in the eastern United States during the past 80 years, it seems unlikely that such spread will occur naturally in the foreseeable future. It may, however, be possible to establish disseminating H strains by knowledgeable intervention.

Perhaps our best hope for enhancing dissemination of H strains lies in an understanding of how hypovirulence spreads where it is spreading (e.g. Italy, France, and perhaps most significantly, western Michigan). The striking similarity between what has happened in Italy and what is happening in western Michigan (Mittempergher 1978; Weidlich this proceedings; Brewer this proceedings) is exciting. Of particular interest is the characteristic lag of about 15 years between V-strain epidemic and the observation of healing cankers. One cannot help but wonder about the source(s) of hypovirulence in these widely separated areas. Intensive studies in these areas should seek to determine the following: rates and patterns of spread; rates of increase of H strains; host density; incidence of vector or other novel relationships affecting dissemination; contribution of asexual and sexual sporulation to dissemination; inoculum density of H strains. With regard to the last point, previous studies by Rogers and Gravatt (1915) and Hebard et al. (1981) have provided some information on the relationship between canker area and rates of disease increase for V strains. Information on how much canker area is required for appreciable spread of H strains is lacking but it probably would be greater than the 500 cm^2/400 m^2 plot reported for V strains (Hebard et al. 1981) because of the reduced sporulation on H cankers.

In concomitant studies in other areas, natural or derived H strains could be evaluated for their capacity to spread. Strains with nuclear (auxotrophic, abnormally pigmented) and/or cytoplasmic (specific component(s) of dsRNA) markers could be utilized for monitoring dissemination. Such studies should take into consideration the possible limitations of vegetative incompatibility, inoculum density, wound susceptibility, and host density.

There is a need for additional information on the basic biology of hypovirulence. Such information could assist in the development of H strains that are most efficient in achieving their assigned task. It is conceivable, for example that certain fragments of genomes of dsRNA permit greater sporulation and carry over of transmissible hypoviurlence in spores.

Slow rates of local spread need not preclude success as H strains can be established artificially in many places throughout the natural range of American chestnut.

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