HYPOVIRULENCE IN ENDOTHIA PARASITICA AND SUGGESTED PROCEDURES FOR ITS DETECTION AND ANALYSIS

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ABSTRACT.--Concepts *of normalcy, virulence, and hypovirulence in Endothia parasitica are presented. Normalcy is defined by the characteristics of a set of four strains (standards) taken from the American and Italian populations of the fungus. Normal virulence is defined by the pathogenicities and reproductive capacities of the standard strains in American chestnut. Hypovirulence is then defined simply as a condition of subnormal virulence. Distinctions are made between two types of hypovirulence, cytoplasmic (CH) and nuclear (NH). These concepts are illustrated with examples of simple and complex hypovirulent strains from the North American and Italian populations of the fungus. Procedures are suggested for the detection and detailed analysis of hypovirulence in individual strains.*

Introduction

What is hypovirulence in the chestnut blight fungus, *Endothia parasitica?* Unfortunately, no precise, operational definition has been published. A narrow concept of hypovirulence is used in most chestnut blight literature and applies only to strains which have low or no pathogenicity and high curative capacity. These strains have been called curative hypovirulent strains to distinguish them from other types of hypovirulent strains (Elliston 1978), but here they will be referred to as 'H' strains. When an 'H' strain is inoculated into healthy bark of European or American chestnut, *Castanea sativa* and *C. dentata,* respectively, it invades, colonizes, and kills little if any tissue. Furthermore, when inoculated into an active blight canker, it rapidly arrests canker development and permits wound closure to proceed. These two properties distinguish 'H' from 'virulent', i.e., wild type or normal strains as clearly as white can be distinguished from black.

In southern Europe, the natural recovery of European chestnut from chestnut blight, which has occurred over the past 30 years, has been attributed to the appearance and natural spread of 'H' strains (Grente and Sauret 1969a; Bonifacio and Turchetti 1973; Mittempergher 1979; Bazzigher et al. 1981). Recovery has been accompanied by a reduction in canker incidence and changes in canker morphology (Grente and Berthelay-Sauret 1978; Biraghi 1950;

Turchetti 1979). The fungus in the abnormal cankers grows more superficially, fruits less abundantly, and stimulates the vascular cambium to lay down additional layers of bark and wood cells, giving the cankers a more or less swollen appearance (Biraghi 1953).

Other 'H' strains of the fungus have been discovered in abnormal cankers on American chestnut trees in areas of western Michigan (Elliston et al. 1977), where natural recovery also is occurring (Brewer this proceedings; Weidlich et al. this proceedings), and on isolated trees in Tennessee (Elliston et al. 1979) and Virginia (Elliston and Kuhlman unpublished data). Presumptive 'H' strains also have been isolated from abnormal cankers on American chestnut trees in other locations within the tree's natural range (Jaynes and Elliston 1982). The elements responsible for the recovery phenomena occurring on the two continents may be manifested in these strains.

Investigators have sought simple, reliable indicators of 'H' to simplify the tasks of detecting these strains in natural populations and studying them in the laboratory and field. Even though deeply pigmented 'H' forms occur (Grente and Sauret 1969b) and have been isolated from nature (Bonifacio and Turchetti 1973), it has been argued that 'whiteness' or slow development of color in culture is a reliable indicator of 'H' (Van Alfen et al. 1978). Double-stranded ribonucleic acid (dsRNA) in the 'white' European 'H' strains and their American derivatives and its apparent absence from 'virulent' European and American strains (Day et al. 1977) suggested that dsRNA might be a reliable indicator of 'H'. However, subsequent studies of a large collection of European and American dsRNA-containing strains (Elliston 1977; 1978 unpublished data) negated 'whiteness' in culture and presence of dsRNA as reliable indicators of 'H'. Wide ranges of cultural abnormalities, levels of pathogenicity, and fruiting capacity occur among these strains. In contrast, characteristics of dsRNA-free European and American strains are much more consistent, as had been reported by earlier investigators (Shear et al. 1917). Many of these observations have been confirmed by Willey (1980). Clearly, a continuum of virulence now occurs in the chestnut blight fungus in nature. We do not yet fully understand what elements are responsible for this virulence continuum, nor do we know how much each element contributes to the recovery phenomena now occurring in southern Europe and western Michigan. The preoccupation with 'H' strains, evident in much of the literature on hypovirulence, may blind us to the possibility that other, less abnormal forms have significant roles. Although curative capacity can be demonstrated most easily with the most debilitated dsRNA-containing strains, these strains could be end products rather than key components of these systems (Elliston 1982). It is possible that other *Endothia* species or even unrelated genera are principal donors of the elements for reduced virulence in *E. parasitica* (Elliston 1982).

A more general concept of hypovirulence may lead to a fuller understanding of the recovery phenomena we are studying. An operational definition of hypovirulence is needed if the revised concept is to be useful. This paper is an attempt to satisfy these needs.

A Revised Concept of Hypovirulence in *Endothia parasitica* and Associated Definitions

The literal definition of hypovirulence probably is most appropriate because it is simple, logical, and has the widest application. The prefix hypo, meaning less than the ordinary or norm (Websters New Collegiate Dictionary 1959), modifies virulence, meaning the disease-producing capacity of an organism (Steen 1971). Therefore, hypovirulence literally means any state of disease-producing capacity less than the norm. This definition merely sets apart all subnormal states of virulence from the normal state; it implies nothing about cause.

This definition of hypovirulence is meaningless without an operational definition for normal virulence, and normal virulence cannot be defined without operational definitions for virulence and normalcy. Many pathologists define virulence as the degree or measure of pathogenicity (Ainsworth 1961; Anonymous 1968; Wood 1967). Pathogenicity can be defined as the capacity to infect, colonize, and disrupt or kill host tissue. Steen's definition of virulence (Steen 1971) offers greater latitude than the common definition. The disease-producing capacity of an organism, in its broadest sense, includes both pathogenicity and reproductive capacity. Reproductive capacity is the pathogen's capacity to produce propagules adapted for dissemination from infected to uninfected tissue within host plant and from individual to individual within a population of the host. This concept of virulence provides the additional criteria needed to distinguish some of the less debilitated strains of *E. parasitica* from normal strains (Elliston 1978). Virulence in *E. parasitica* then, has four measurable components: amount of bark surface area colonized, depth of infection, number of mature pycnidia, and number of mature perithecia at one or more specified intervals after superficial inoculation of American or European chestnut. Depth of infection is included because strains that penetrate rapidly from the outer layers of bark to the vascular cambium and sapwood are more damaging, and therefore more virulent, than strains that colonize the same volume of bark tissue in the same time interval but grow more superficially. Assessment of both pycnidium and perithecium production is included because strains capable of producing both conidia and ascospores have greater disease-producing capacity than strains capable only of producing conidia. Although precise measurements of depth of infection and numbers of mature fruiting bodies would require extensive destructive sampling, rough estimates of these might suffice in most situations.

Normal strains of the chestnut blight fungus will be defined as those which are homokaryotic, i.e., have one type of nucleus, are free of infectious agents, and have characteristics typical of the species (Elliston 1978). This definition has meaning only if a standard set of strains is designated and used for comparison, and these strains are in their normal, vigorous physiological state when used. Degree of vigor usually is evident from cultural characteristics if one has had sufficient experience with the fungus. If in comparative tests a strain differs significantly from the standard strains in one or more characters, it is abnormal with respect to those characters. When that character is virulence, strains not significantly different from the standards have normal virulence, those with significantly lower virulence are hypovirulent, and those with significantly higher virulence are hypervirulent. A strain can have normal virulence and be abnormal in one

or more other characters (this would be an abnormal strain with normal virulence). Also, normal strains in culture often assume one of several transient, physiologically abnormal states in which their virulence is subnormal (these would be normal strains with transient hypovirulence).

These definitions apply at the species level. In practice, analysis of hypovirulence begins with individual strains taken from the natural population. Thus, there also is a need for a concept of normalcy at the level of the individual strain. This is especially true when a strain is encountered that is atypical of the species. Two types of strains can be distinguished: simple strains, which are homokaryotic, and complex strains, which are heterokaryotic, i.e., have two or more types of nuclei. The 'normal' state of a simple strain will be defined as that in which it is free of infectious cytoplasmic agents and growing most vigorously. Normal virulence for such a strain is its level of virulence when in this state. The strain is hypovirulent with respect to its normal state when its virulence is significantly lower. These definitions apply to complex strains only if the strain is stable. Otherwise these states cannot be defined. All states of an abnormal strain could be hypovirulent with respect to the standard normal strains.

Distinctions between types of hypovirulence are needed and might best be founded on whether the genetic information that determines them is nuclear or cytoplasmic. Nuclear hypovirulence (NH) is due to nuclear genetic determinants (NH agents) and might be caused by mutant nuclear genes, genes brought together in heterokaryons, hybrid nuclei, or extrachromosomal genetic determinants that reside in nuclei. Nontransmissible hypovirulence (Van Alfen this proceedings) may be a synonym for NH. Cytoplasmic hypovirulence (CH) may be conditioned by agents (CH agents) such as viruses and virus-like pathogens, plasmids, and organelles, such as mitochondria, that carry genetic elements. Since many of these agents also fit the definition of a hyperparasite, CH often can be viewed as a consequence of hyperparasitism, or more properly, hyperpathogenism. Synonyms that have been used for CH include 'exclusive' hypovirulence (Grente and Sauret 1969a), contagious hypovirulence (Grente 1975), transmissible hypovirulence (Van Alfen et al. 1975), infectious hypovirulence (Kuhlman 1980), and hypovirulence (Jaynes et al. 1976; Day et al. 1977).

It is probable that in some strains NH and CH agents act together to reduce virulence (Elliston 1982; unpublished data). The intensely pigmented strains from Europe, the 'JR' type of Grente and Sauret (1969b) and the 'P' type of Bonifacio and Turchetti (1973), behave as if they have abnormal nuclear conditions and contain virus-like CH agents (Elliston unpublished data). Rare, intensely pigmented single conidial isolates which are free of CH agents have been obtained from these strains. They fail to transmit CH agents into compatible normal strains unless they are first allowed to interact with compatible strains containing these agents. The subnormal virulence of the slow-growing, white European CH strains may be due to heterokaryosis and infection with virus-like CH agents. These strains typically yield complex mixtures of single conidial isolates. These include: typical normal; slow-growing, intensely pigmented; highly variable, unstable, slow-growing white; and fast-growing white types. The variable, slow-growing 'white' types, like the parent strain, probably are heterokaryons infected with more than one virus-like CH agent. Another strain, EP-405, from central Italy behaves as if it

contains at least three types of nuclei, based on the pattern of segregation of its conidia and the behavior of each of the types.

In Figure 1, several hypothetical strains are used to illustrate many of these concepts.

Suggested Procedures for Detection and Detailed Analysis of Hypovirulence

The detailed analysis of a strain for hypovirulence usually begins by comparing its cultural characteristics with those of two or more standard strains of *E. parasitica.* Strains EP-155, 408, 421, and 523, selected from the 22 dsRNA-free strains used in the study of selected CH agents (Elliston this proceedings), are now being used as standards in this laboratory. The origins of these four strains are given in Table 1 of that paper. Cultural characteristics are determined on 100 x 15 mm plastic disposable petri dishes containing 20 ml of Difco PDA amended with 0.1 g L-methionine and 0.1 mg biotin per liter (PDAmb). Preparing and dispensing this medium with a model AS-3 agarmatic bench-top agar sterilizer fitted with a model M1062 dispensing pump helps ensure uniformity. Inoculum for determining cultural characteristics consists of 7 mm diameter plugs of mycelium and agar cut with a sharp, sterile cork borer from the advancing margins of actively growing, vigorous colonies. The plugs are oriented with the mycelium side up. Three replicate plates of each strain are each sealed with a layer of parafilm and incubated with a 16 hr photoperiod at 20 C, 75 cm beneath banks of fluorescent lights spaced 25 cm apart. Cultures are examined when 7- to 9-days old. If the cultural characteristics of the test strain(s) are consistently different from those of the standards, the analysis proceeds. If the results are inconclusive, the comparative experiment is repeated. If the test strain is indistinguishable from the standards, it probably is normal. If its virulence is normal and it lacks detectable dsRNA, it would not be studied further.

If a strain has abnormal cultural characteristics, single conidial isolation experiments are conducted to determine if more than one type of single conidial isolate (SCI) is obtained. In each experiment, all germlings from two plates of complete medium (Puhalla and Anagnostakis 1971) containing approximately 50 germlings each, are transferred, cultured, and compared with standard normal strains and the parent strain. If only one type of isolate is obtained, the parent strain either is a mutant, a member of another species, or a CH strain in which the CH agent enters most of the conidia it produces. The SCI experiment would be repeated. If the same results are obtained, the strain probably would be tested for virulence in American chestnut, presence of dsRNA, transmissibility of the abnormality directly or indirectly to the standard normal strains, and capacity to mate with mating type testers of *E. parasitica* (Anagnostakis 1979), and, if perithecia are produced, ascospore progeny would be examined for the pattern of segregation of morphological types. The course of further analysis would depend upon the outcome of these tests.

If in the SCI experiment more than one isolate type were obtained, isolates representing each type would be retained and tested for virulence, stability in SCI experiments, presence of dsRNA, transmissibility to the most normal appearing type of SCI, and transmissibility directly or indirectly to the standard normal strains. If one of the SCI types is stable in further SCI

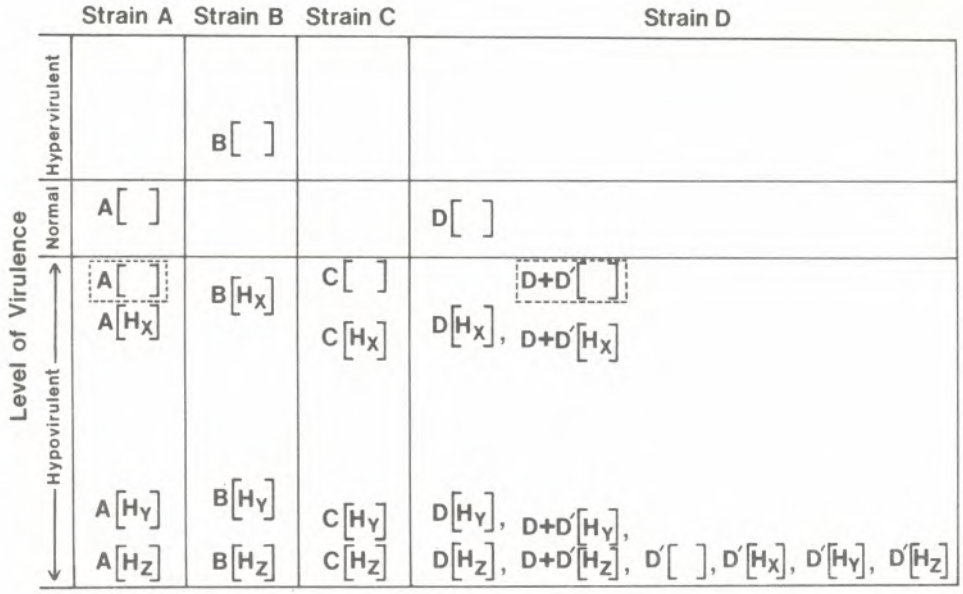

Figure 1. Four hypothetical strains of *Endothia parasitica* which illustrate many of the concepts discussed in the text. Each strain is presented in a variety of states, each having a different level of virulence. Each state is represented by one or more uppercase letters, A, B, C, D, or D', which represent different genotypes, followed by a set of brackets containing information about the state of the cytoplasm. A single letter signifies a homokaryon; more than one letter signifies a heterokaryon. Empty brackets indicate normal, uninfected cytoplasm. Symbols Hx , and H represent three different virus-like cytoplasmic hypovirulence (CH) agents which, when present in a strain, have mild, moderate, and severe effects on virulence, respectively. Only single infections are shown. Sets of symbols enclosed by dashed lines represent the strain in a state of transient hypovirulence. The normal level of virulence for the species is estimated using the four standard strains. Strain A, when uninfected with CH agents, has normal virulence, i.e., virulence typical of the species as estimated with the standards. It is shown in a state of transient hypovirulence and in three states of cytoplasmic hypovirulence. Strain B, when uninfected, is hypervirulent, i.e., more virulent than normal. It displays nuclear hypervirulence. It too is shown in three states of cytoplasmic hypovirulence. Strain C, when uninfected, is hypovirulent, i.e., it is less virulent than normal. It displays nuclear hypovirulence. When strain C is infected with one of the CH agents, it is in a state of cytoplasmic hypovirulence. Strain D is a complex strain containing two types of nuclei, D and D'. An isolate of strain D containing only the D type nucleus, and having uninfected cytoplasm, has normal virulence. When such an isolate is infected with a CH agent, the strain has cytoplasmic hypovirulence. An isolate containing only the D' type nucleus has severe nuclear hypovirulence. Its level of virulence is the same whether or not it is infected with CH agents. The heterokaryon, D+D', is unstable in the absence of CH agents. The normal nucleus and the CH agent largely determine the level of virulence of the heterokaryon.

experiments, lacks detectable dsRNA, and has some resemblance to typical *E. parasitica, it* is assumed to be the normal state of the strain. Its virulence is compared with that of the standard strains to determine if it is typical of the species.

The number of distinct abnormal SCI types obtained from the parent strain provides a clue to the number of elements present. If the parent strain consistently yields only two distinct SCI types, one of which resembles the parent and the other is dsRNA-free and has normal or near normal cultural characteristics, the parent probably contains a single agent conferring the abnormality. If the abnormality can be transmitted from the parent strain to the most normal SCI type, the agent probably is a cytoplasmic determinant. If the parent strain and the abnormal SCI type like it contain dsRNA with the same pattern, the parent probably is a CH strain containing one virus-like CH agent. If the abnormality and dsRNA are transmissible to one or more of the standard normal strains, this conclusion almost certainly is valid. The effect of the CH agent on the cultural characteristics and virulence of the standard strains can then be determined and compared with those of cultures of standard strains containing other CH agents.

If more than two SCI types are obtained from the parent strain, more than one agent is present, and the analysis becomes more involved. The parent strain could be a simple strain containing two or more CH agents or a complex strain containing two or more types of nuclei and one or more CH agents. Examples of some of the simple and more complex situations encountered to date in our laboratory and their tentative or predicted analyses are presented in the next section.

Tests for virulence can be conducted at any point in the analytical procedure but usually are not made until the degree of complexity of the situation has been estimated from SCI experiments, preliminary transmissibility experiments, and dsRNA analyses. Tests can be conducted using excised, dormant trunk sections of American chestnut trees with smooth bark and with living trees with smooth bark in the field (Elliston 1978). Excised stem tests are useful for detecting very weak to moderately weak pathogenicity and for estimating capacity to reproduce asexually. Field tests are preferred because they permit a much more thorough assessment of virulence. Three or four of the standard normal strains should be tested along with one or more test strains or, preferably, a test strain and one representative of each of the SCI types obtained from it. It is important that all strains in a test be inoculated into each tree because highly significant differences have been found in canker development in different trees but not within individual trees, so long as smooth bark is inoculated and the basal region of the tree is avoided (Elliston this proceedings; unpublished data). The Latin square experimental design is ideally suited for these experiments. Also, trees 10 cm or more in diameter at 1.4 m are preferred because tests then can be carried well into the second growing season. This is desirable because the hypovirulence of some strains is not very evident until the second growing season (Elliston unpublished data). Although Anagnostakis and Waggoner (1981) found rate of canker expansion during one growing season useful for comparing short term pathogenicities of strains and mixtures, the many observations required make this approach impractical for long term tests.

Ideally, inoculations should be superficial and made in May or early June, using inoculum taken from the advancing margins of actively growing, vigorous colonies. If inoculum is used from old cultures or from colonies in one of the transient abnormal states referred to earlier, infection often fails or canker development is retarded or otherwise abnormal (Elliston unpublished data). A minimum of five observation dates are recommended unless the test strain is markedly hypovirulent. The first observations should be made in late July or early August (canker area, degree of superficiality, and abundance of stromata and pycnidia), the second in late November or early December (canker area, degree of superficiality, abundance of stromata with perithecia), the third in late March or early April (canker area, degree of superficiality, abundance of stromata with perithecia), the fourth in late July or early August of the second growing season (canker area, degree of superficiality, abundance of new stromata with pycnidia), and the fifth in late November or early December of the second season (canker area, degree of superficiality, abundance of new stromata with perithecia). This set of observations should give an estimate of virulence for the test strain(s) and SCI. To determine the effects of these agents in strains typical of the species, similar tests would be conducted with sets of infected and uninfected standard strains.

If it is not clear from the virulence test that a strain is *E. parasitica*, this can be determined by a mating type test (Anagnostakis 1979).

Examples of Simple and Complex Hypovirulent Strains from Nature

Figure 2 illustrates the tentative analyses of three simple strains, EP-234, 418, and 60. Figure 3 is the predicted analysis of EP-419, and Figure 4 is a partial analysis of EP-405. Both EP-419 and EP-405 are complex strains. Strain EP-234, from an abnormal canker on *C. dentata* in Tennessee, contains one virus-like CH agent, HT_2, which severely weakens virulence. Strain EP-418, from an abnormal canker on *C. sativa* in southern Tuscany, Italy, contains one virus-like CH agent, H12, which prevents perithecium formation and stops canker development after the first growing season. Strain EP-60, from an abnormal canker on *C. dentata* in western Michigan, contains two virus-like CH agents: HM_1, which severely weakens virulence, and HM2, which weakens virulence considerably but does not prevent formation of perithecia and normal ascospores. When present together in a strain, effects of HM_1 are dominant. Strain EP-419, from the same canker as EP-418, appears to contain nuclei of two types, CH agent H12, and probably one or more other virus-like CH agents (Figure 3). Finally, strain EP-405, from an abnormal canker on *C. sativa* in central Tuscany, Italy, appears to contain nuclei of three types and one virus-like CH agent similar to H_{12}. Other even more complex strains, e.g., EP-90 from Michigan, have been encountered, but they have not yet been analyzed.

The complex Italian CH strains, such as EP-419 and EP-405, are intriguing. Accumulated evidence suggests that they might arise from simple strains infected with $H1_2$, or other CH agents like it, by processes of 'degeneration'. Degeneration may result from nuclear mutation, to yield a less virulent heterokaryon, combined with mutation of the CH agent to yield a mixture of closely related CH agents. The cytoplasm of strains infected with certain CH agents may permit heterokaryons to persist. The degeneration of H12-infected strains to the $H1_1$-infected state, which has occurred occasionally

in the laboratory, may be due to these mutations. The hypothesis for this process of degeneration is depicted in Figure 5. A process such as this may be occurring in chestnut blight cankers in Italy and may be responsible for their gradual deactivation. If so, the HI_2-type CH agents may be the key CH agents in the natural recovery process occurring in Italy.

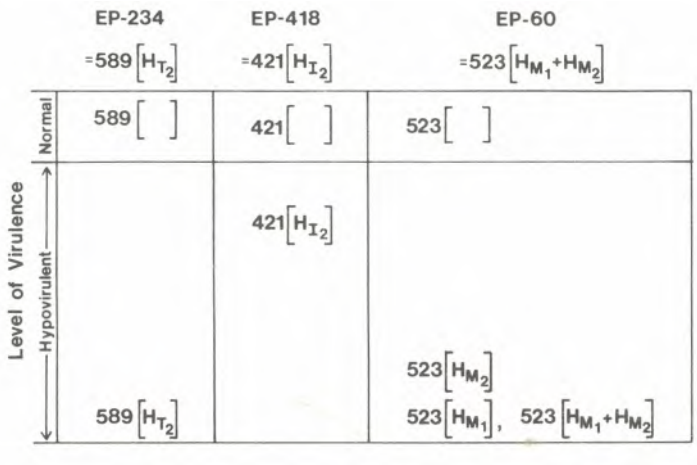

Figure 2. Tentative analyses of three simple CH strains of *Endothia parasitica*. Strain EP-234, from Bonair, Tennessee, is a normal strain, EP-589, containing one highly debilitating CH agent, HT2. Strain EP-418, from Mt. Amiata, Tuscany, Italy, is a normal strain, EP-421, containing one weakly debilitating CH agent, HI2. Strain EP-60, from Rockford, Michigan, is a normal strain, EP-523, containing two CH agents, Hmi, which is highly debilitating, and HM2, which is moderately debilitating. The effects of Hm_1 dominate when both agents are present.

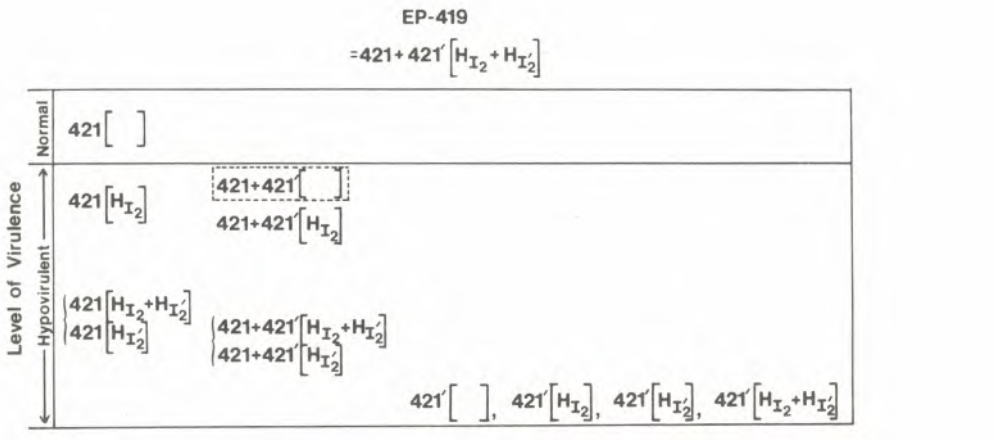

Figure 3. Predicted analysis of EP-419, a strain obtained from the same bark sample as EP-418 (see Figure 2), EP-419 probably is a complex strain containing normal type nuclei, represented by EP-421, abnormal nuclei, represented by EP-412 [1], and two closely related CH agents, H12 and H121. Infection states preceded by a brace presumably have the same virulence level.

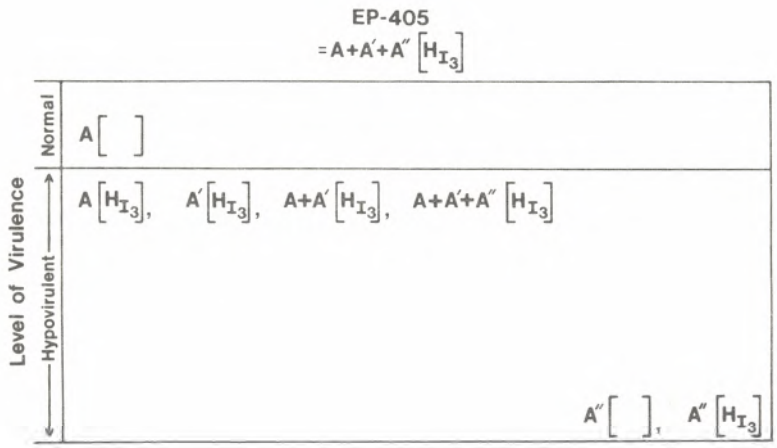

Figure 4. Partial analysis of strain EP-405 from Mt. Senario, Tuscany, Italy. It appears to be a complex strain containing normal nuclei, A, two abnormal types of nuclei, A', and A'', and one weakly debilitating CH agent, H_{I3}. Many probable infection states are not shown.

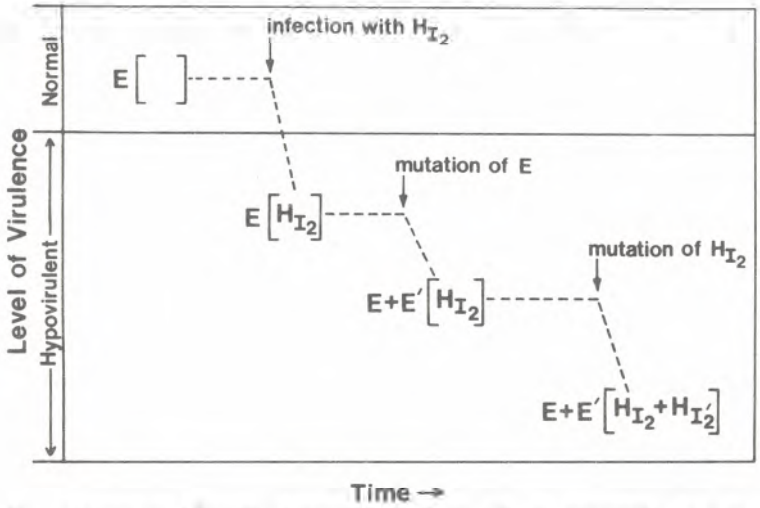

Figure 5. Hypotethical degeneration of a normal strain of *Endothia parasitica* after infection with CH agent H_{I2}.

Discussion

In *E. parasitica* growth and morphogenesis, i.e., development of form, and virulence are highly sensitive to changes in internal and external conditions. It is well known to anyone who has worked with this fungus that normal strains, i.e., those typical of the species, are quite sensitive to differences in growth medium, temperature, light intensity, and light periodicity. Perhaps we should be surprised that a strain of *E. parasitica* can be described as 'typical'. Yet, early investigators of chestnut blight marvelled at the high degree of uniformity among the thousands of cultures of this fungus that they examined (Shear et al. 1917).

These considerations, in addition to the high, rather uniform susceptibility of European and American chestnut to blight, probably account for the discovery of 'H' in this fungus. The first '-' strains discovered in Europe and North America were isolated from abnormal cankers, have markedly low levels of virulence, highly abnormal cultural characteristics, and display high levels of curative capacity. Just because these strains and their unusual properties were discovered first is not a valid reason to confine the concept of hypovirulence in *E. parasitica* to them. The apparently continuous ranges of abnormalities found in the pathogenicity, reproductive capacity, and cultural characteristics of dsRNA-containing strains suggest that many agents may be involved. It appears also that abnormal nuclei might contribute to the reduced virulence of some strains.

The concept of hypovirulence suggested here accommodates all forms of subnormal virulence and classifies them according to cause. Such a concept provides a rationale for the detailed analysis of the causes of reduced virulence in individual strains. The procedures suggested for these analyses are tedious and time consuming, but they are necessary if we are to develop a fuller awareness and understanding of the hypovirulence phenomenon in *E. parasitica*.

Literature Cited

Ainsworth, G. C. Dictionary of the fungi. 5th ed. Kew, Surrey, England: Commonwealth Mycological Institute; 1961. 547 p.

Anagnostakis, S. L. Sexual reproduction of *Endothia parasitica* in the laboratory. Mycologia 71:213-215; 1979.

Anagnostakis, S. L.; Waggoner, P. E. Hypovirulence, vegetative compatibility, and the growth of cankers of chestnut blight. Phytopathology 71:1198-1202; 1981.

Anonymous. Plant pathologists' pocketbook. Kew, Surrey, England: Commonwealth Mycological Institute; 1968. 267 p.

Bazzigher, G.; Kanzler, E.; Kubler, T. Irreversible pathogenitats verminderung bei *Endothia parasitica* durch ubertragbare hypovirulenz. Euro. J. For. Path. 11:358-369; 1981.

Biraghi, A. Caratteri di resistenza in *Castanea sativa* nei confronti di *Endothia parasitica*. Boll. Staz. Patol. Veg.:VII, III 5:167-171; 1950.

Biraghi, A. Ulteriori notizie sulla resistenza di *Castanea sativa* Mill. nei confronti di *Endothia parasitica* (Murr.) And. Boll. Staz. Patol. Veg. XI:149-157; 1953.

Bonifacio, A.; Turchetti, T. Differenze morphologiche e fisiologiche in isolati di *Endothia parasitica* (Murr.) And. Ann. Accad. Ital. Sci. For. 22:111-131; 1973.

Day, P. R.; Dodds, J. A.; Elliston, J. E.; Jaynes, R. A.; Anagnostakis, S.L. Double-stranded RNA in *Endothia parasitica*. Phytopathology 67:1393-1396; 1977.

Elliston, J. E. Abnormalities in morphology, growth, and virulence in *Endothia parasitica* containing double-stranded RNA. Proc. Amer. Phytopath. Soc. 4:111; 1977.

Elliston, J. E. Pathogenicity and sporulation of normal and diseased strains of *Endothia parasitica* in American chestnut. MacDonald, William L.; Cech, Franklin C.; Luchok, John; Smith, Clay, eds. Proceedings of the American chestnut symposium; 1978 January 4-5; Morgantown, WV. Morgantown; West Virginia University Books; 1978: 95-100.

Elliston, J. E. Hypovirulence. Advances in Plant Pathology 1: (in press); 1982.

Elliston, J. E.; Jaynes, R. A.; Day, P. R.; Anagnostakis, S. L. A native American hypovirulent strain of *Endothia parasitica.* Proc. Am. Phytopath. Soc. 4:83; 1977.

Elliston, J. E.; McCarroll, D.; Wooding, B. Hypovirulent strains of *Endothia parasitica* discovered in Tennessee. IX Int. Cong. Plant Protection, Abstr. 633; 1979.

Grente, J.; Sauret, S. L'hypovirulence exclusive, phenomene original en pathologie vegetale. C. R. Acad. Sc. Paris; D; 268:2347-2350; 1969a.

Grente, J.; Sauret, S. L'hypovirulence exclusive, est-elle controlee par des determinants cytoplasmiques? C. R. Acad. Sc. Paris; D; 268:3173-3176; 1969b.

Grente, J. La lutte biologique contrala chancre du chataignier par "hypo-virulence contagieuse". Ann. Phytopath. 7:216-218; 1975.

Grente, J.; Berthelay-Sauret, S. Biological control of chestnut blight in France. MacDonald, William L.; Cech, Franklin, C.; Luchok, John; Smith, Clay, eds. Proceedings of the American chestnut symposium; 1978 January 4-5; Morgantown, WV. Morgantown: West Virginia University Books; 1978: 30-34.

Jaynes, R. A.; Elliston, J. E. Hypovirulent isolates of *Endothia parasitica* associated with large American chestnut trees. Plant Disease (in press) 1982.

Jaynes, R. A.; Anagnostakis, S. L.; Van Alfen, N. K. Chestnut blight fungus. J. E. Anderson and H. Kaya, eds. Perspectives in forest entomolygy. New York: Academic press; 1976. p. 61-70.

Kuhlman, E. G. Hypovirulence and hyperparasitism. J. G, Horsfall and E. B. Cowling, Plant Disease, Volume V; 1980. p. 363-380.

Mittempergher, L. The present status of chestnut blight in Italy. MacDonald, William L.; Cech, Franklin C.; Luchok, John; Smith, Clay, eds. Proceedings of the American chestnut symposium; 1978 January 4-5; Morgantown, WV, Morgantown: West Virginia University Books; 1978: 34-37.

Puhalla, J. E.; Anagnostakis, S. L. Genetics and nutritional requirements of *Endothia parasitica.* Phytopathology 61:169-173; 1971.

Shear, C. L.; Stevens, N. E.; Tiller, R. J. *Endothia parasitica* and related species. 1917; USDA Bull. 380. 82 p,

Steen, E. B. Dictionary of biology. New York: Barnes and Noble; 1971. 630 p.

Turchetti, T. Prospettive di lotta biologica in alcune malattie di piante forestali. Informatore Fitopatologico 29:7-15; 1979.

Van Alf en, N. K.; Jaynes, R. A.; Anagnostakis, S. L.; and Day, P. R. Chestnut blight: biological control by transmissible hypovirulence in *Endothia parasitica.* Science 189:890-891; 1975.

Van Alfen, N. K.; Jaynes, R. A.; Bowman, J. T. Stability of *Endothia parasitica* hypovirulence in culture. Phytopathology 68:1075-1079; 1978.

Websters New Collegiate Dictionary. Springfield: Merriam; 1 1,174 p.

Willey, R. L. Pathogenicity and sporulation of selected hypovirulent and virulent strains of *Endothia parasitica.* Morgantown: West Virginia University; 1980; 111 p. Masters Thesis.

Wood, R. K. S. Physiological plant pathology. Oxford: Blackwell Scientific Publications; 1967. 570 p.

Acknowledgement

The excellent technical assistance of Ms. Barbara Wooding is gratefully acknowledged and greatly appreciated.