HOST-PARASITE INTERACTIONS OF *ENDOTHIA PARASITICA* ON CHESTNUT SPECIES: STATE OF THE ART

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ABSTRACT.--Cankers incited by *Endothia parasitica* on American, European, Japanese and Chinese chestnut trees have been described for various parts of the world and may be classified into four general types: A) sunken, B) irregularly swollen, C) callused and swollen ((i) with and (ii) without necrosis of the callus bark tissue), and D) superficial and swollen. Intermediate forms among A to D have been described and more than one canker type may be observed on the same tree. Canker types Cii or D have been associated commonly with virulent E. *parasitica* on Chinese and Japanese chestnut trees and hypovirulent E. *parasitica* on American and European chestnut trees, whereas canker types A, B or Ci are commonly associated with virulent E. *parasitica* on American, European and sometimes on Japanese and Chinese chestnut trees. Environmental factors (cold injury, frost injury, low soil fertility, shading or competition, and low soil water potential) that stress the host appear to play central roles in increasing the development of virulent E. *parasitica* on the normally blight-resistant Oriental chestnut species. The extent and nature of wound periderm formation appear to be important factors limiting E. *parasitica* infections in canker types Cii and D, but wound periderm appears to be ineffective, in most instances, in canker types A, B and Ci, due to a rapid growth rate of mycelial fans of the pathogen in bark tissues. In American and European chestnut trees, wound periderm may not be formed or is often not fully formed when the virulent pathogen invades this and neighboring tissues. Tannins (especially hamamelitannin in *Castanea dentata* and C. *sativa*), carbohydrates and amino acids in bark tissues appear to be key energy, carbon and nitrogen sources supporting rapid E. *parasitica* mycelial fan growth. The mechanical forces exerted by the mycelial fan, oxalic acid toxicity, acidification of host tissues (partly through the production of oxalic acid), and hydrolytic enzymes may play important roles in pathogenesis by E. *parasitica*.

The pioneering histopathological and histochemical research of Keefer (1914) and Bramble (1936) laid the foundation for our understanding of the host-parasite interactions of Endothia parasitica on American chestnut Castanea

dentata. Subsequent work by Bazzigher (1955; 1957) of a histopathological and physiological nature provided new insights and permitted a comparison of the behavior of *E. parasitica* on European *C. sativa* and American chestnut trees. Today, hypovirulence in *E. parasitica* and blight resistance in Chinese chestnut *C. mollissima* offer contrasting systems to determine the key factors limiting canker development on chestnut, in hope that this understanding will enable us to better control virulent forms of the pathogen.

Cankers incited by *E. parasitica* on *C. dentata, C. sativa,* Japanese chestnut, *C. crenata* and *C. mollissima* have been described for various parts of the world (Biraghi 1953; Bramble 1936; Bonifacio and Turchetti 1973; Heald 1913; Headland et al. 1976; Jones et al. 1980; Mittempergher 1978; Uchida 1977) and may be classified into four general types:

A) Sunken. Cankers rapidly expanding with a sunken and commonly split surface. Infection extends to the vascular cambium, and death is typically observed. Fructifications are typically abundant, particularly on smooth bark. Often the canker margin is slightly swollen or raised on American, European and Japanese chestnuts.

B) Irregularly swollen. Cankers with irregularly swollen and sunken areas due to progression and cessation of lesion and host tissue growth. Splitting and sloughing of the bark may occur. Infection often extends to the vascular cambium. Fructifications are abundant to sparse on American, European, Japanese and Chinese chestnuts.

C) Callused and swollen. Cankers characterized by early progressive infection of bark tissues to the vascular cambium with one or more prominant callus (differentiated xylem and phloem) ridges ringing the deep infection, and with (i) or without (ii) invasion or necrosis of the callus tissues by *E. parasitica*. Exposed wood may be present at the canker center and on the older callus ridges that give a swollen appearance to the stem. Infected tissues may slough completely away in some instances. Fructifications are sparse to moderately frequent on American, European, Japanese and Chinese chestnuts.

D) Superficial and swollen. Cankers with superficial infection of small or large areas of the bark that typically give a swollen appearance to the bole or branch. Little or no infection extends to the vascular cambium in most instances. There may be extensive sloughing of infected tissues. Fructifications are sparse to nonexistent. Large canker areas may be observed on American and European chestnut trees and small areas may be observed on Chinese and Japanese chestnut trees.

Intermediate forms among A to D have been described and more than one canker type may be observed on the same tree.

Canker types Cii or D have been associated with virulent *E. parasitica* on Chinese and Japanese chestnut trees and hypovirulent *E. parasitica* on American and European chestnut trees, whereas canker types A, B or Ci are commonly associated with virulent *E. parasitica* on American, European and sometimes on Japanese and Chinese chestnuts.

In a study conducted over 13 provinces of Italy, Bonifacio and Turchetti (1973) isolated normal (virulent) strains of *E. parasitica* from 9 of 13

restricted (=healed) or partially restricted cankers on *C. sativa.* Nine of 13 restricted or partially restricted cankers yielded morphologically atypical strains that were hypovirulent. From four of 13 cankers of these two types only atypical strains were isolated. All of nine nonrestricted cankers yielded normal strains and seven of these yielded only normal strains. All of four restricted cankers yielded at least one atypical strain type. Thus, restricted cankers were generally, although not exclusively, associated with the presence of atypical strains.

Mittempergher (1978) distinguished two types of restricted cankers in Italy, which correspond to the superficial swollen (type D) and callused swollen types (type Cii) above. Bark swelling and low pycnidium production were associated with both. The superficial cankers covered large areas of the stem. The bark was slightly cracked and appeared rough and dark in color. Cork or wound periderm appeared to effectively restrict *E. parasitica* colonization of tissues toward the vascular cambium and contributed to the sloughing of infected tissues. The second canker type exhibited *E. parasitica* infections to the vascular cambium, and mycelial fans were evident on the xylem. Wound responses were observed in swollen areas surrounding the deep infection and these restricted further canker development. Presumably, the latter was due to the infection of bark tissues by a hypovirulent strains(s) subsequent to the infection of the vascular cambium by a virulent strain(s).

Restricted or, more commonly, partially restricted cankers have been observed by us on large, surviving and stump-sprout American chestnut trees in the Appalachian region of the United States. Some of these cankers correspond to the healthy, callused-swollen (type Cii) or superficial-swollen type (type D), but most correspond to the necrotic, callused-swollen type (type Ci) according to our observations. Sometimes an individual tree will have all three canker types. The presence of the latter type may be associated with death of that portion of the stem or tree if invasion of the callus and inner bark is extensive. Crown ratings (percent of branches not killed) for surviving American chestnut trees over 25 cm d.b.h. are commonly over 50 percent. For one such tree in northern Virginia we found approximately one-third of 100 bark-tissue isolates of *E. parasitica* were hypovirulent in inoculation trials on American chestnut stump sprouts. Superficial swollen cankers were common on this tree, as indicated by examination of corkborer bark samples from the extensively cankered stems. Lower percentages (10 to 30 percent) of hypovirulent *E. parasitica* were found in 12 other surviving American chestnut trees and associated smaller American chestnut trees in northern Virginia, based on trials with 10 bark-tissue *E. parasitica* isolates per tree. In an earlier study (Griffin et al. 1978), using one isolate per tree, and in a more recent study, using 30 isolates from a single West Virginia tree, we found little association of hypovirulent *E. parasitica* with partially restricted cankers on large, surviving American chestnut trees. Jaynes (1981) surveyed 20 large (mostly > 30 cm d.b.h.), surviving American chestnut trees and found that many of the selected abnormal isolates from these trees were hypovirulent. Further research is required on determining the levels of *E. parasitica* hypovirulence and blight resistance in such trees before we can make a reliable assessment on how much hypovirulence contributes to the survival of large, surviving American chestnut trees. Through inoculation trials with virulent *E. parasitica,* we have found that several surviving trees have various levels of blight resistance that may be a factor in survival. Trees with superficial swollen cankers or callused swollen cankers

are the most likely candidates for this research. Small superficial cankers are found on blight-resistant Chinese chestnut, from our observations, and Uchida (1977) has described cankers on normally blight-resistant Japanese chestnut that may be of this type. He reports that the necrotic tissue is often sloughed off from these cankers.

Wound periderm formation is typically associated with successful attempts by chestnut species to limit infection by both virulent and hypovirulent *E. parasitica,* and appears to be a principal tissue, along with phloem parenchyma, that is associated with swollen, superficial cankers on chestnut boles or branches. As indicated above, the bark swelling feature is often associated in the same canker with sunken or killed bark tissues or with exposed wood tissues, even in blight-resistant Chinese chestnut. Histopathological studies by Bramble (1936) on American chestnut and by Bazzigher (1957) on European chestnut, infected with virulent *E. parasitica,* indicate wound periderm, composed of about eight cork-cell layers in the phellem, is effective in blocking colonization by *E. parasitica.* However, Bramble (1936) noted that complete infection of American chestnut bark by virulent *E. parasitica* was associated with the absence of wound periderm formation. Both Bramble (1936) and Bazzigher (1957) observed that the virulent fungus could advance in bark tissues containing wound periderm in areas where wound periderm had not yet formed. Similarly, Grente and Berthelay-Sauret (1978) reported that small islands of cork cells, not forming a continuous barrier, were formed in European chestnut bark tissues infected with virulent strains. With hypovirulent *E. parasitica* strains, a complete cork barrier was observed. These observations raise the question as to whether the rate of wound periderm formation, the completeness of wound periderm formation or the thickness of wound periderm formation are important in restricting canker development. The lignified zone, or wound periderm-induction barrier (WPIB), does not limit infection by the pathogen (Bramble 1936). To examine the above aspects, Hebard et al. (1979) inoculated highly blight-resistant Chinese chestnut ('Nanking'), a moderately blight-resistant, surviving American chestnut in Virginia and blight-susceptible American chestnut stump sprouts with a killing strain of *E. parasitica* and with hypovirulent (slightly and weakly pathogenic) strains of the fungus. They found few differences in the initiation time for the formation of the WPIB or wound periderm, among the different trees and for inoculated and noninoculated wounds, in the secondary phloem over the first 10 to 20 days. Thereafter, maturation and completeness of wound periderm formation lagged in the secondary phloem and cortex of blight-susceptible stump sprouts inoculated with the virulent strain, and the formation of wound periderm was less uniform for this combination. In many instances no wound periderm was formed in the blight-susceptible trees. For all chestnut types, wound periderm formation occurred first in the secondary phloem near the vascular cambium and developed later in the cortex or outer bark tissues. For moderately blight-resistant American chestnut inoculated with virulent *E. parasitica,* wound periderm in the secondary phloem and cortex blocked further infection by the fungus, except in the cortex where there was a small or large discontinuity in the three- or four-cell thick wound periderm or where a mycelial fan had apparently pushed laterally through a two-cell thick wound periderm. In Chinese chestnut inoculated with the virulent strain, the fungus was restricted, although not always, by wound periderm that formed as rapidly, as completely and as thickly (about eight cells) in the inoculated wounds as in the noninoculated wounds. With a few exceptions, no gaps or thin wound periderm areas were

noted in the secondary phloem and cortical wound periderm. Similar results were found for the slightly pathogenic Italian hypovirulent strain EP-66, on highly blight-resistant Chinese chestnut and moderately blight-resistant American chestnut, but this strain on blight-susceptible American chestnut was associated with the formation of significantly fewer cell layers in the wound periderm, compared to the noninoculated control or other low-disease, pathogen-host combinations.

Research by Hebard et al. (1979) has suggested that the dominant factor in host-parasite interactions of a progressive, lethal canker is the rapid formation and growth of the mycelial fan. The wedge-shape mycelial fan of the virulent pathogen on blight-susceptible American chestnut appears to exert a physical pressure that the lignified zone or tissues other than a many-cell-layered wound periderm cannot withstand (Bramble 1936; Hebard et al. 1979; Keefer 1914). Splitting of bark tissue in front of the fan was commonly observed by Hebard, Griffin and Elkins (unpublished data). In addition, cell death occurred at least 350 pm in advance of the fan in the absence of a lignified zone, as indicated by neutral red straining. If fans were close to the newly forming lignified zone or WPIB, this death may have prevented wound periderm formation. Histopathological observations made by Hebard et al. suggest this may occur. Grente and Berthelay-Sauret (1978) have suggested the *E. parasitica* toxin, diaporthin, may prevent wound periderm formation, but McCarroll (1978) found no evidence that diaporthin was present in cankered tissues on American chestnut. McCarroll (1978) proposed that oxalic acid production and acidification of host tissues by *E. parasitica* played important roles in pathogenesis in front of the mycelial fan. It is possible that oxalic acid and other acidic agents may interfere with wound periderm formation after the mycelial fan breaks through the lignified zone. McCarroll (1978) reported there was a drastic decline in the pH of bark tissues associated with recent infection. He found that the pH values of bark tissues in the uninfected areas, the gelatinous zone ahead or the fan (probably the brown or yellow-brown zone described by other workers), and advancing edge of mycelium were 5.5, 4.7 and 2.8, respectively. Oxalic acid concentration increased with infection and was 3.8 to 1.1 mg/g bark in uninfected areas and 9.3 mg/g bark in area of the gelatinous zone and leading 0.5 cm of mycelium. In bioassays, oxalic acid caused a browning of cells near the vascular cambium.

Hebard, Griffin and Elkins (unpublished data) observed that virulent *E. parasitica* produced multiple, rapidly growing mycelial fans in susceptible American chestnut stump sprouts and one was frequently located at the vascular cambium. In contrast, slower-growing fans were formed by two slightly pathogenic hypovirulent isolates (American W-2 and Italian EP-66), and none were observed for two weakly pathogenic hypovirulent isolates (EP-14 and EP-3) in blight-susceptible American chestnut. In moderately blight-resistant American chestnut and highly blight-resistant Chinese chestnut, typically only one fan was formed by virulent *E. parasitica,* and this was located in the outer bark tissues where, as mentioned, wound periderm formation occurred later. The combination of these two factors (one fan formation and late wound periderm formation in the cortex) appear to be the key elements to explain the superficial nature of many cankers caused by virulent *E. parasitica* on blight-resistant chestnut trees and by hypovirulent *E. parasitica* on blight-susceptible chestnut trees. In the latter instance, the slower rate of fan formation and growth of the hypovirulent isolate, compared to the

virulent isolate, may permit more complete formation of wound periderm in the secondary phloem next to the vascular cambium. In the cortex, where wound periderm formed later, the pathogen is able to advance superficially. No mycelial fans were formed by hypovirulent *E. parasitica* on moderately or highly blight-resistant chestnut trees in the Hebard et al. study.

The rapid growth and multiple formation of mycelial fans by virulent *E. parasitica* in blight-susceptible American chestnut may be dependent in part upon the high availability of utilizable tannins in this species. Other carbon- and nitrogen-containing energy sources, such as carbohydrates and amino acids, are likely to be important as well. Although tannins may be toxic at high concentration (Nienstaedt 1953; Uchida 1977), Cook and Wilson (1916); Bazzigher (1955) and Uchida (1977) demonstrated that *E. parasitica* utilized the tannins of American, European and Japanese chestnut trees, respectively. Significantly, Bazzigher (1955) found that tannins were removed from European chestnut bark during canker development. Elkins et al. (1978) found that utilization of tannins from aqueous bark extracts of American, European and Chinese chestnut trees was greater for the two blight-susceptible species than for the blight-resistant species. Similarly, the mycelial growth (dry weight) of *E. parasitica,* on which fan formation is dependent, produced on extracts of bark was greater for the two blight-susceptible species than for the blight-resistant species. A pH drop following growth, possibly due to oxalic acid production, was observed for all extracts. Elkins et al. (1979) found that bark tissues of blight-susceptible American and European chestnut trees contained high amounts of hamamelitannin whereas none was found in blight-resistant Chinese and Japanese chestnut trees. Hamamelitannin was rapidly utilized for growth by *E. parasitica* in axenic culture.

Bramble (1936) made observations that suggest the browning reaction at the margin of the mycelial fan may be due to tannin oxidation or to polymerization of tannins. At the margin of the fan, the color intensity of the host cells increased initially, upon treatment with FeC13, which was followed by no color when the cell contents had turned yellow-brown. Both he and Hebard, Griffin and Elkins (unpublished data) also observed a greater FeC13 reaction in the area of wound periderm formation, suggesting mobilization of phenolics by the host in that area. Biochemical defense mechanisms in blight-resistent chestnuts may be important also in limiting fan formation in blight-resistant chestnuts. McCarroll (1978) demonstrated that Chinese chestnut tissues displayed a greater sensitivity to oxalic acid and acidification than American chestnut and suggested that hypersensitivity (as indicated by browning) of Chinese chestnut to these agents may be a factor in blight resistance. He suggested that pathogen enzyme denaturation and pectinase inhibition by the host were also important factors.

Environment appears to be a dominant factor in host-parasite interactions of virulent *E. parasitica* on blight-resistant Chinese and Japanese chestnut trees, but little is known about the effect of environment on the interaction of hypovirulent *E. parasitica* and blight-susceptible American and European chestnut trees. Uchida (1977) has shown that low soil fertility (especially nitrogen), low soil water potential, shading or competition, frost injury or cold injury were important in increasing the susceptibility of Japanese chestnut to *E. parasitica.* He found also that seedlings were more susceptible than older trees. Observations of Berry (1951) and surveys of Jones et al.

(1980) in the United States suggested that frost injury or cold injury or both were associated with increased susceptibility of Chinese chestnut trees to *E. parasitica.* Thus, environmental factors that stress the host appear to be important factors in affecting host-parasite interactions. These stress factors may affect the ability of the host to rapidly form a continuous and fully developed wound periderm or to effect biochemical defense. In both large, surviving American chestnut trees and Chinese chestnut trees, we have observed, following canker dissection, instances where the advance of the pathogen in the bark was restricted, except where small radially advancing "leads" of necrotic tissue had progressed deeply below the generally contained infection. Similarly, in these trees we often observed a local area of infection at the vascular cambium, as evidenced by small areas of exposed wood or necrosis of the vascular cambium at the canker center. Apparently, this occurred under conditions stressful to the host. Subsequently, the host formed callus tissues, with differentiated xylem, phloem and wound periderm that excluded the pathogen, when conditions became more favorable to the host. As stated previously, often a series of four or five exposed xylem ridges may be observed for cankers on surviving American chestnut trees or on Chinese chestnut trees, suggesting a periodicity of environmental conditions unfavorable and favorable to defense against *E. parasitica.* Using regression analysis, Uchida (1977) found the extent of callus development by Japanese chestnut (probably an indicator of tree vigor) was inversely related to canker development. In American or European chestnut infected with hypovirulent strains, differences in the ability of the host to limit infection of *E. parasitica* in an individual canker also may be a reflection of the relative proportion of virulent or hypovirulent thallus in the canker. Areas in the canker where necrotic "leads" extend beyond the generally limited canker may be areas where virulent strains are present.

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