EXPERIMENTATION WITH HYPOVIRULENT *ENDOTHIA PARASITICA* IN MICHIGAN

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ABSTRACT.--Samples *of Endothia parasitica obtained from American chestnut trees in Michigan have been isolated in pure culture according to standard techniques. Isolates from virulent populations and presumed hypovirulent populations of Michigan E. parasitica are presently in culture. Michigan virulent strains of E. parasitica are normal in appearance and cause cankers on excised chestnut wood that are similar in size to those reported elsewhere. Michigan American chestnut trees with healing or quiescent cankers have been tested for susceptibility to virulent forms of E. parasitica. The response of these Michigan trees suggests that they are not resistant to disease. Strains of E. parasitica from healing stands appear morphologically normal or abnormal in culture. These strains of E. parasitica possess dsRNA that is capable of being transferred to vegetatively compatible virulent strains of E. parasitica in culture and on excised dormant chestnut wood. Field tests were initiated in the spring of 1981 to determine the possibility of using native Michigan strains of hypovirulent E. parasitica to control virulent populations of E. parasitica in Michigan. The results of laboratory tests and field trials are presented.*

Most of the *Castanea dentata* occurring in Michigan has been established outside its natural range by early orchard fruit growers. There are 600 to 800 individuals 61 cm to 152 cm diameter and thousands more if smaller trees are taken into account. *Castanea dentata* occurs primarily in the west and northwestern portions of the lower peninsula in groves with 7 to 3,000 trees. The distribution of *C. dentata* is not continuous and the groves can be regarded as islands semi-isolated from each other in terms of reproduction and in terms of the strains of *Endothia parasitica* they may harbor.

In relation to *E. parasitica*, *C. dentata* exists in three situations: *(1)C. dentata* that remain free of *E. parasitica* and resultant disease; *(2)C. dentata* infected with *E. parasitica* that causes "normal" disease symptoms; and (3) *C. dentata* infected with strains of *E. parasitica* causing abnormal, healing cankers.

Endothia parasitica has been present in Michigan since the late 1920's and has gradually been spreading across the state. Presently, few groves remain free of *E. parasitica.* We are of the opinion that those few stands of *C. dentata* remaining free of chestnut blight have simply avoided the disease. We consider some stands to harbor virulent forms of *E. parasitica* because the disease spreads rapidly and causes complete destruction of the periderm and secondary phloem with resultant adventitious shoots developing below the girdling canker. Numerous stromata protrude from the diseased bark producing pycnidia and perithecia and eventual death of the infected tree occurs.

Eight of the 10 stands of *C. dentata* we have visited have two types of abnormal, healing cankers. The first type was initially normal in that it caused complete destruction of the periderm and secondary phloem. The remains of old mycelial fans can be seen on the surface of the secondary xylem. At the same stage of canker development all further growth of the canker ceased and the tree began healing over the quiescent canker (Figure 1A). The second type of abnormal canker is completely superficial (Figure 1B).

Figure 1. Types of healing cankers in Michigan. A. Healing canker that was initially expanding but now appears in remission with new chestnut tissue encroaching over the margins of the canker. B. Superficial canker completely encircling the stem.

A superficial canker may completely encircle a stem but girdling does not occur. Adventitious roots do not develop below the canker. Hand sections have revealed that the mycelium is confined to the periderm and does not produce broad mycelial fans. Stromata are rare and we have observed no perithecia or pycnidia. Both types of abnormal cankers appear to be similar to healing cankers on *C. sativa* in Italy. Healing cankers in Michigan appear to lead to a gradual improvement in the health of individual trees and entire stands.

A stand of *C. dentata* in Grand Haven, Michigan, is an excellent example of trees with healing cankers. According to the property owner, chestnut blight was apparent in the stand by 1945 and initially caused severe damage. Presently the disease is almost completely in remission with little yearly dieback. The property owner maintains that the health of the trees has improved over the last 15 to 20 years. To check the unlikely possibility that these trees may have resistance to *E. parasitica*, we removed dormant branch wood from three individuals and tested it in the laboratory with four virulent strains of *E. parasitica* and one strain from Grand Haven. Table 1 shows the sizes of developed cankers after 5 weeks incubation. The developed cankers

Table 1. Canker development after five weeks incubation on excised Grand Haven dormant chestnut wood.

Endothia parasitica strain number	Canker size
	mm^2
69	1591
59	1093
89	1752
CL4	1226
351	1420
GH2	787

are smaller than those reported elsewhere, but probably do not indicate that these trees are resistant. The excised branches though small (approximately 10 cm diameter) were quite old and had a well-developed rhytidome as opposed to a simple periderm found in younger branches of similar size. A more mature bark may slow the tangential spread of *E. parasitica.* Young excised wood from other stands of *C. dentata* with healing cankers showed more normal response to virulent *E. parasitica* (Table 2).

Sixteen isolates of *E. parasitica* obtained from bark samples collected at Grand Haven were established in pure culture as mass isolates in Difco potato dextrose agar (PDA). In culture, these Grand Haven strains more clearly resembled typical virulent strains in terms of growth rate, pigmentation and sporulation. The virulent strains used for comparison are a group from Eastern North America supplied to us by Anagnostakis and a group isolated from a virulent infection at Crystal Lake, Frankfort, Michigan.

Pathogenicity tests were performed using these 16 strains of Grand Haven *E. parasitica* on excised dormant chestnut wood from a single coppice group. These 16 strains represent a large sample of *E. parasitica* population at Grand Haven. Seven virulent strains were used as a control. The results are presented in Table 2. There is considerable variation in canker size. The size of cankers from some Grand Haven isolates overlapped with the size of cankers produced by the virulent strains, however, when the canker sizes produced by Grand Haven *E. parasitica* are averaged and then compared with the average for the virulent strains, the average canker produced by the

Grand Haven *E. parasitica* population is approximately one-third of the average canker produced by the tested virulent strains.

Table 2. Canker development by Grand Haven strains of *Endothia parasitica* compared to "virulent" *E. parasitica* on dormant chestnut wood. The same wood was used for each strain.

"Virulent"		Grand Haven	
E. parasitica strain number	Canker size	*E. parasitica* strain number	Canker size
	mm^2		mm^2
351	7200	2	571
59	2858	4	595
69	1440	5	1344
89	4468	6	1747
CL1	4333	7	2371
CL2	4733	8	1196
CL4	2707	U1	2176
Average	3963	U2	1440
		U3	986
		U4	96
		12	880
		1B	2240
		A	1500
		14	1240
		17	121
		3	1815
		Average	1270

We suspected that this reduction in capacity for disease by the Grand Haven *E. parasitica* may be due to transmissible hypovirulence. We tested Grand Haven strain 2 (GH2) and Grand Haven strain U4 (GHU4) for the presence of dsRNA. The GHU4 is an abnormal strain morphologically in culture and is nearly avirulent (Table 3). The GH2 appears normal in culture and produces a somewhat larger canker on excised wood (Table 3). Both of these strains have dsRNA as determined by polyacrylamide gel electrophoresis.

Table 3. Canker development by CL1, GHU4, and converted CL1 on dormant chestnut wood.

Endothia parasitica strain	Canker size	Average
	mm^2	mm^2
CL1	2655, 3500, 2160	2772
GHU4	100, 64, 0, 49, 64, 96	62
Converted CL1	0, 0, 51	17

Grand Haven U4 converts the morphological and pathogenic characteristics of Crystal Lake strain 1 (CL1) (Table 3). This conversion is correlated with the transfer of dsRNA from GHU4 into CL1 and occurs 100 percent of the time on PDA and in excised chestnut wood. The CL1 and GHU4 appears to be vegetatively compatible thus accounting for the 100 percent conversion frequency. The GHU4 converts the morphological and pathological characteristics of CL4 approximately 30 percent of the time on PDA. In the other 70 percent, a barrage indicative of an incompatible reaction is produced between the strains when paired on PDA. Conversion may not be the result of a complete and long lasting anastomosis between two vegetatively compatible strains. When CL4 is converted by GHU4, no barrage is produced. Genetic incompatibility lack between GHU4 and CL4 is not a barrier to the transfer of dsRNA since GHU4 converts CL4 most of the time on excised dormant chestnut branches.

Crystal Lake 1 and CL4 were isolated from a large grove of 3,000+ C. *dentata* (3,000 or more trees) at Crystal Lake, Frankfort, Michigan. This stand is presently infected by a virulent population of *E. parasitica.* Since in the laboratory we were able to convert CL1 and CL4 with GHU4, we decided to initiate a field experiment to determine if we could control CL1 and CL4 with our stock of "compatible" hypovirulent strains. The CL1 and CL4 were used to establish multiple cankers on disease free saplings 13 to 18 cm diameter by using the cork borer technique. The cankers were allowed to develop for 5 weeks and were challenged by one of four hypovirulent strains. Figure 2 shows a conversion and/or vegetative compatibility matrix between the Crystal Lake virulent strains and the Grand Haven hypovirulent strain. The GHU4, GH2, converted CL1 and converted CL4 were used to challenge the established CL1 and CL4 cankers. Single virulent cankers were established to replicate each challenge 12 to 15 times. The CL1 and CL4 cankers were allowed to develop for 5 weeks before challenging and placing mycelia from the appropriate hypovirulent strain into four cork bore holes around each canker. The challenges to CL1 cankers were started on June 4, 1981 and the challenges to the CL4 cankers were started on July 2, 1981. Measurements of the CL1 and CL4 cankers were made prior to challenging. Four bore holes were also placed around the control canker but no hypovirulent inoculum was placed in the holes.

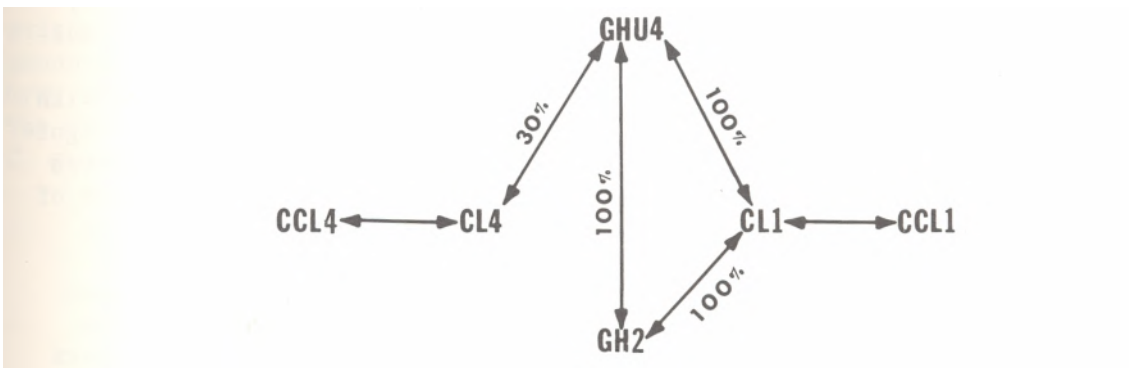

Figure 2. Compatibility and/or conversion matrix between two Grand Haven hypovirulent strains and two Crystal Lake virulent strains.

In mid-October, the cankers were measured to determine the difference in size, if any, among the cankers challenged with the four hypovirulent strains, compared to the control cankers. Figure 3 presents this data for the CL1 control and the hypovirulent challenges to CLI. The data for CL4 are similar.

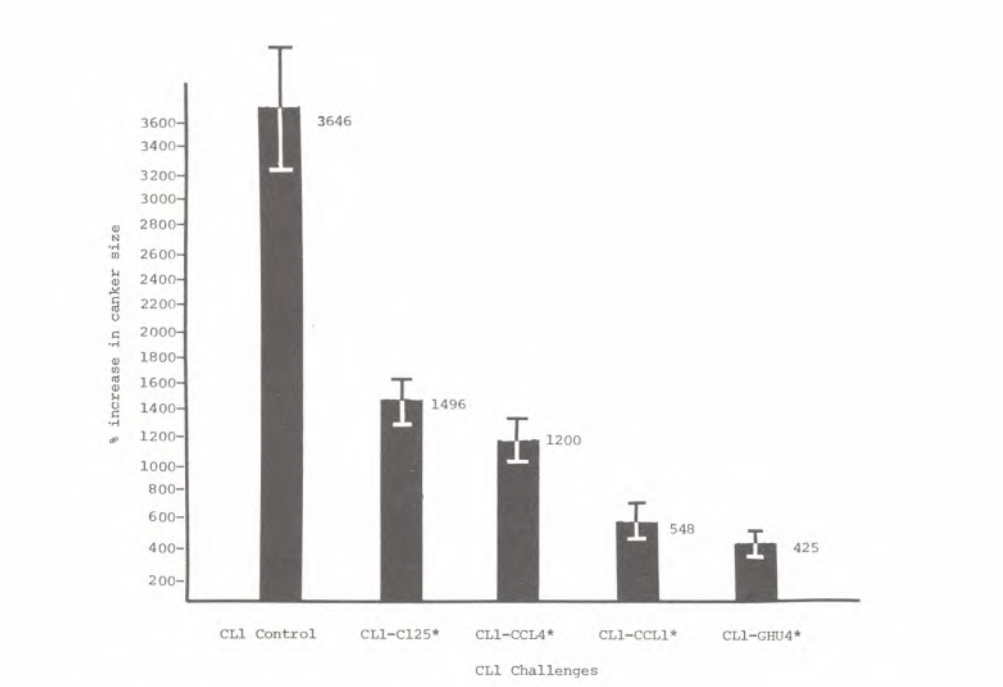

Figure 3. Percent increase in growth of control CL1 cankers and the challenge CL1 cankers during the summer of 1981. Bars equal one standard error of the mean challenging strains.

but this experiment has not run long enough for us to be certain of the results. The bar graph represents the percent increase in canker size (mm^2) from the time of challenge to the final canker measurement in October. During this time interval, the size of the unchallenged CL1 control cankers increased by an average of 3,646 percent whereas the cankers challenged with the hypovirulent strain GHU4 converted CL1 and converted CL4 increased significantly less. Some size increase with the challenged cankers was expected because the hypovirulent strains caused a canker of their own around each of the four bore holes. Some CL1 cankers were challenged with *E. parasitica* strain CL25 isolated from a canker at the Crystal Lake grove. The small percentage of canker size increase is probably due to inhibition or competition between these two strains. Crystal Lake 25 does not possess dsRNA. The differences in size increase between the control canker and the cankers challenged with our native hypovirulent strains should increase dramatically over time if a conversion has taken place on the trees.

We were not content to wait a couple of years to see if we could just control these small cankers we initiated. Presently, about 20 percent of the trees in the Crystal Lake grove have at least one expanding canker. We

decided to challenge 36 of the large existing cankers with our four new hypovirulent strains of *E. parasitica.* Each canker was challenged with a single hypovirulent strain by ringing the margin of the canker with cork borer holes. The borer holes were filled with the appropriate mycelia and PDA and were covered with masking tape. These challenges were initiated on June 4, 1981. At the same time, we collected bark samples from each canker and established pure cultures of *E. parasitica* from each canker. Every isolate appeared normal in culture. We will use these cultures as references for what *E. parasitica* strain was causing the canker. Later if the challenged cankers go into remission, we can sample for possible conversion and transfer of dsRNA. Shortly after establishing the challenge to the cankers, we outlined the margin of other existing cankers that we did not challenge. This was done so that we could compare the growth of a normal canker on these medium sized trees.

Thirty-three of the 36 challenged cankers ceased expanding for the growing season. Healthy new host tissue was present at the margins of the same challenged cankers. These cankers resembled the early stages of healing cankers elsewhere in Michigan that were initially virulent and became converted naturally. The new tissue at the margins of the cankers appeared free of *E. parasitica.* We sampled the margins and centers of these cankers for *E. parasitica* and were able to obtain some cultures that were morphologically abnormal in culture as are the hypovirulent strains used for the challenge.

All the hypovirulent challenging strains that we used produced very small cankers (under 100 mm^2) when inoculated alone in *C. dentata.* These strains may control individual cankers but they may be too debilitated as pathogens to persist, spread and establish additional hypovirulent cankers and hypovirulent inoculum. In mid-summer, we found dsRNA in GH2. The GH2 produces a persistent long-lived canker on *C. dentata* in the Grand Haven grove and produces a relatively small canker with spore horns on excised dormant *C. dentata* wood. We are presently evaluating the ratio of normal to hypovirulent conidia produced by GH2. Grand Haven 2 strain was used to challenge a large established Crystal Lake canker on July 26, 1981. The GH2 may be an appropriate native hypovirulent strain to establish at Crystal Lake because it produces a long-lived persistent superficial canker at Grand Haven. Such a hypovirulent canker could be an important source of hypovirulent inoculum. The somewhat increased virulence of GH2 seems to be within the tolerance of C. *dentata* in Michigan.