A Preliminary Report on a Method of Biological Control of the Chestnut Blight Not Involving the Use of a Hypovirulent Strain of *Endothia parasitica*

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ABSTRACT.—A method of eradicating active cankers of *Endothia parasitica* from specimen trees of *Castanea dentata* by the application of soil compresses is described. Evidence is presented that an interaction between an isolated but unidentified soil fungus and *E. parasitica* is responsible for remission of cankers following an application of a soil compress to the infected area.

Early in the 20th century it was realized that the chestnut blight, caused by *Endothia parasitica* (Mum) P. J. & H. W. And., was a serious threat and if left unchecked *Castanea dentata (Marsh.*) Borkh. would be eliminated as a forest tree. In light of this threat, a number of methods to control the spread of the disease or to at least nullify its effects were pursued by various workers. These approaches involved quarantine and clean up of diseased chestnut individuals, the aseptic surgical removal of cankers, the application of fungicide to infected trees, searching for and breeding resistant C. *dentata* individuals, and the production of hybrids between American chestnut and blight resistant oriental chestnut species. Initially, these approaches seemed to hold great promise, but as time went on it became frustratingly apparent that there were no quick solutions for restoring a blight resistant chestnut timber tree to the forests of North America. Recently, Van Alfen *et al.,* (1975) described a method of biological control of the chestnut blight through the use of hypovirulent strains of *Endothia parasitica.* It was hoped that chestnut blight in North America could be controlled as it has been in Europe. However, it became apparent that the biology of host-parasite relationship is different in North American chestnut stands than in chestnut stands in Europe (Anagnostakis, 1979; Day, 1979; Grente, 1979) and it is too early to predict the total effectiveness of hypovirulent strains of *E. parasitica* in controlling chestnut blight in North America.

This paper is a preliminary report describing a method of eradicating existing *E. parasitica* cankers on American chestnut without the use of hypovirulent strains of *E. parasitica.* For the study situation it was found that this method could be

used to keep specimen chestnut trees alive until superior control methods such as hypovirulence or the production of blight resistant American chestnuts can be developed.

THE APPLICATION OF SOIL COMPRESSES TO CHESTNUT TREES

Below ground portions of chestnut coppice groups do not normally develop cankers (Hepting, 1974). In 1963, I noticed cankers developing on chestnut roots following their exposure to the air during the construction of a logging road. This fact plus the observation that cankers present on the bases of chestnut trees fail to develop more than a centimeter below the ground level suggested that soil may exert an inhibitory effect on the growth of *E. parasitica* cankers. A treatment of active cankers with soil from around the base of the infected tree was found to be effective in causing cankers to go into remission allowing recovery of the infected individual.

Materials and Methods for the Application of a Soil Compress

A soil compress was made by gathering soil from around the base of an infected chestnut tree and mixing it with enough water to cause it to become muddy. A polythylene bag was then secured well below and around an existing canker with tape or other suitable material. The bag was filled with the muddy soil and secured at the top with tape to prevent the soil compress from drying. The entire canker was well covered with soil which should extend beyond the visible area of infection. The soil compress remains in place for at least two months or preferably an entire growing season. If a tree has developed multiple cankers and if the stem is under five inches in diameter, a metal stove pipe can be fitted around the stem and filled with muddy soil. A canker at the base of a tree was treated by simply mounding soil around the base of the tree.

Results

From 1963 to 1975 approximately 50 American chestnut trees in New Hampshire and Massachusetts were treated with soil compresses. In every case the application was effective in the remission of cankers if the compress was properly applied. Application of air-tight polyethylene bags without soil or the application of roofing tar as an air-tight barrier instead of a soil compress were not effective. The growth of cankers was accelerated in the moist environment of the polythylene bag without soil. The application of roofing tar did not affect the rate of canker spread. *E. parasitica* stroma simply erupted through the tar.

In May, 1975, the canker in Figure 1 was treated with a soil compress (Fig. 2). At that time the tree was approximately 85 percent girdled by the canker. The soil compress was removed in mid-August, 1975. The treatment resulted in the remission of the canker and the development of callus tissue along the edges of the canker (Fig. 3, at arrow).

These results are typical of the soil compress technique. If the active canker is completely covered with soil and left intact for three or more months, it will be destroyed. Subsequently, the canker does not become active unless reinfection occurs.

Figure 1. Active *E. parasitica* canker on an American chestnut sapling. The stem is approximately 85 percent girdled.

Figure 2. Soil compress in place over the canker pictured in Fig. 1.

Figure 3. Canker following treatment with soil compress. Arrow indicates area of callusing over the inactive canker.

EVIDENCE SUGGESTING A BIOLOGICAL INTERACTION BETWEEN *E. PARASITICA* AND A SOIL FUNGUS

With the success of a soil compress in treating active cankers of *E. parasitica* reasonably well documented, the next step was to determine if the physical presence of the soil of influence or a soil organism caused remission of cankers.

Materials and Methods

E. parasitica was cultured in the laboratory on PDA (potato dextrose agar), on PDA plus 10 percent autoclaved soil, and on PDA plus 10 percent unautoclaved soil. Inoculations of *E. parasitica* were made by placing a 5 mm^2 piece of inoculum in the center of petri plates prepared as described above. The inoculated petri plates were then placed in the dark at 25 C. Measurements of the growth of *E. parasitica* in mm on PDA, PDA plus 10 percent unautoclaved soil, and PDA plus 10 percent autoclaved soil were made daily for a week.

The various soil organisms that could grow aerobically on PDA were isolated by serial dilution and were maintained in axenic culture. The isolated soil organisms were tested for possible inhibitory affects on the growth of *E. parasitica* by culturing *E. parasitica* and a particular soil isolate together on opposite sides of a petri plate containing PDA.

Results and Discussion

The growth of *E. parasitica* on PDA, PDA plus 10 percent unautoclaved soil, and PDA plus 10 percent autoclaved soil is presented in Table 1. The growth of *E. parasitica* on PDA was interpreted as being normal. At the end of eight days the culture of *E. parasitica* grown on PDA completely filled the petri plates (Table 1) and produced a normal growth curve (Fig. 4). The growth rate of *E. parasitica* on PDA plus 10 percent autoclaved soil slightly lagged behind the growth rate of *E. parasitica* on PDA (Table 1). However, a normal growth curve resulted on PDA plus 10 percent autoclaved soil that paralleled the growth curve of *E. parasitica* on PDA (Fig. 4). The differences in growth rate on PDA and PDA plus 10 percent autoclaved soil could simply be due to the fact that the cultures set up in 10 percent autoclaved soil contained 90 percent PDA whereas the *E. parasitica* cultures set up on PDA grew on 100 percent PDA. The 10 percent dilution of PDA in PDA plus autoclaved soil may have reduced the available nutrients enough to account for the slower growth rate of *E. parasitica* on PDA plus 10 percent autoclaved soil. However, the possibility of a slight inhibitory effect of the 10 percent autoclaved soil on the growth of *E. parasitica* cannot be ignored.

Growth of *E. parasitica* on PDA plus 10 percent unautoclaved soil did not take place (Table 1). At the end of eight days on PDA plus 10 percent unautoclaved soil *E. parasitica* failed to develop beyond the 5 mm^2 inoculum (Table 1, Fig. 4). The

Table 1
The effect of autoclaved and unautoclaved soil on the growth of *Endothia parasitica* on PDA

	SIZE OF *ENDOTHIA PARASITICA* COLONY (mm)																	
Day	Control (PDA)				PDA + Percent Unautoclaved Soil								PDA + 10 Percent Unautoclaved Soil							
Plate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2	14	17	15	15	0	0	0	0	0	0	0	0	10	10	14	10	10	12	13	10
3	25	27	25	26	0	0	0	0	0	0	0	0	13	18	*	24	24	24	24	23
4	38	43	39	40	0	0	0	0	0	0	0	0	33	36	*	37	31	36	35	28
5	50	54	54	51	0	0	0	0	0	0	0	0	43	40	*	45	45	52	48	44
8	90	90	90	90	0	0	0	0	0	0	0	0	85	80	*	85	80	85	85	85

*Contaminated

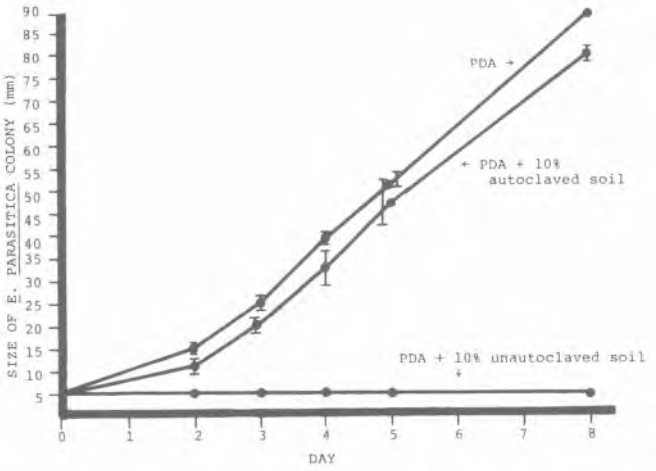

Figure 4. Growth of *E. parasitica* on PDA, PDA + 10 percent autoclaved soil, and PDA + 10 percent unautoclaved soil. Bars indicate three times the standard error of the mean.

original inoculum of *E. parasitica* appeared moribund or dead. The petri plates containing PDA plus 10 percent unautoclaved soil were contaminated by a number of organisms.

Fifty-three different organisms were isolated from the soil sample and maintained in sterile culture on PDA. The isolated organisms consisted of an assortment of fungi, actinomycetes, and bacteria. Each one of these isolates was tested for possible inhibitory effects on the growth of *E. parasitica* on PDA. Isolate #46 was extremely active in inhibiting the growth of *E. parasitica.* Isolate #46 is a fungus that is possibly a *Trichoderma* species (tentative identification made by S. L. Anagnostakis).

When isolate #46 and *E. parasitica* were placed on separate sides of a petri plate, the two mycelia grew towards each other at approximately the same rate on PDA. When the mycelium of isolate #46 approached within 5-7 mm of the advancing *E. parasitica* colony, further advancement of *E. parasitica* mycelium ceased. Eventually the hyphae of isolate #46 grew over the mycelium of *E. parasitica.* As the hyphae of isolate #46 grew over *E. parasitica,* a distinct band of killed *E. parasitica* 5-10 mm wide was produced in advance of the isolate #46 hyphae. *Endothia parasitica* could not be reisolated from this band.

Apparently, isolate #46 produces a water soluble inhibitor that is capable of diffusing 5-10 mm in PDA. However, it is possible that the growth of isolate #46 may alter the PDA culture conditions, such as changing the pH, in such a way as to arrest the development of *E. parasitica* without the production of an inhibitor.

CONCLUSIONS

The application of a soil compress is a useful technique for preserving specimen trees of *Castanea dentata.* The compress destroys existing cankers, but does not protect the tree from reinfection and additional canker development later. Although this technique is awkward, it is effective.

One of the difficulties in grafting chestnut is the cutting of the stock and scion which results in the strong possibility of infection by *E. parasitica* st the graft wound. If the graft is successful and is initially free of the blight, it is still prone to infection. The developing callus tissue with its fissured bark is extremely susceptible to infection and canker development. The use of a soil compress instead of or in addition to grafting wax would protect the graft union from infection by *E. parasitica.* If a graft union develops a canker at some later date the soil compress can be applied at that time. A soil compress could thus maintain a potentially valuable scion. The scion for instance may be from an American chestnut that expresses some degree of natural resistance. The soil compress technique could keep the scion alive long enough for it to reproduce sexually. At such a time the scion could be used in hybridization experiments. A soil compress could preserve potentially valuable chestnut genomes.

The soil compress technique also has useful application in chestnut hybridization studies. For example, if an American chestnut or a hybrid were found with some degree of natural resistance to *E. parasitica,* such an individual could be preserved with soil compresses until pollen or seed were collected. If a tree were cankered but surviving and one wanted seed or pollen from it, it could be treated with a soil compress if a canker threatened the pollen or seed crop. The soil compress technique is not the answer for treating the chestnut blight in forest trees. Its use is limited to the preservation of specimen trees until natural resistance can be bred into the natural population or until the control of chestnut blight through hypovirulence can be perfected.

Culture experiments with added autoclaved or unautoclaved soil indicate that there is a living component of soil or a heat labile compound that prevents the growth of *E. parasitica* in culture on PDA. Isolates of organisms taken from a sample of soil used in Figure 2 led to the discovery of a fungus species that first inhibits the growth of and eventually kills *E. parasitica* when the two fungi are placed in the same petri plate. This fungus has tentatively been identified as a *Trichoderma* species. Thus evidence so far indicates that a *Trichoderma* species that is a part of the fungal soil flora from around the base of chestnut trees may be the active agent responsible for the success of the soil compress technique in the treatment of cankers caused by *E. parasitica.*

In the future I will continue my work along the following lines:

1. Refinement and further experimentation with the soil compress technique.
2. Isolate #46 will be positively identified as to species.
3. The presence or absence of an inhibitor will be investigated.

4. Cultures of isolate #46 on a convenient medium will be used directly against active cankers.

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