American Experience With Hypovirulence in *Endothia parasitica*

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ABSTRACT.— A general overview of the hypovirulence research program at the Connecticut Agricultural Experiment Station is presented. Through a team effort the group hopes to make rapid progress to find the best way to use hypovirulence for the biological control of chestnut blight in the United States.

Any discussion of American experience with hypovirulence in chestnut blight properly begins, I think, in the 1930's when Robert Frost wrote a poem:

> Will the blight end the chestnut?
> The farmers rather guess not.
> It keeps smoldering at the roots
> And sending up new shoots
> Till another parasite
> Shall come to end the blight.

Our work on hypovirulence began in 1972 with cultures very kindly sent to us by J. Grente. American chestnut seedlings were inoculated in the greenhouse with French and American virulent strains of *Endothia parasitica* (Murr.) P. J. & H. W. And., with French hypovirulent strains, and as pairs with each other. We found that the French strains alone and in pairs behaved as described by Grente (1965) and Grente and Sauret (1969a, b) (Anagnostakis and Jaynes, 1973). The two pairings of an American virulent strain with a French hypovirulent were not clear. One of the trees died and the other showed extensive fungal growth, but no wilting, even after 100 days. The tree wound was heavily calloused and isolations of *E. parasitica* were made from this tissue before the trees were sterilized to satisfy plant quarantine requirements. In retrospect, our choice of strains was far from ideal. The reisolated strain looked like the original French hypovirulent when grown on agar media in the lab, and single conidia spread on agar yielded a variety of colony morphologies, as was reported for the original (Grente and Sauret, 1969a, b). In 1972, our work was done under a plant quarantine permit which did not allow field tests, but since our results looked promising, we were granted permission in 1973 to conduct experiments on field grown trees at our Experiment Station farm. Neal Van Alfen and Richard Jaynes made many paired inoculations of American virulent strains with the reisolated hypovirulent strain and got better disease control than we had first experienced. A blight canker on a field grown tree usually has a shrunken appearance when the tree has been girdled by fungal growth. A canker inoculated with a hypovirulent strain stops enlarging, and the tree forms callus, giving the wound a swollen appearance. When one of these healed cankers is sampled around the periphery the recovered strains are usually predominately hypovirulent.

Tests with strains identifiable by nuclear genes (Puhalla and Anagnostakis, 1971) proved that hypovirulence is determined by genes in the cytoplasm of *E. parasitica* and that these determinants are transferred from strain to strain in the host and

on agar media in the lab. Biochemical tests run by Peter Day revealed the presence of double-stranded ribonucleic acid (dsRNA) in the cytoplasm of hypovirulent strains but not virulent strains. This is the genetic material of most fungal viruses. All of this work was published (Van Alfen, *et al.,* 1975) and we turned our attention to American chestnut trees in the forest. Richard Jaynes tested 42 kinds of native and exotic woody plants for susceptibility to disease caused by virulent or hypovirulent strains of *E. parasitica.* These included plants from 17 different families. The only plants showing any growth of the fungus were American chestnut *(Castanea dentata* [Marsh.] Borkh.), "Crane" Chinese chestnut (C. *mollissima* Bl.), "Eaton" chestnut *(C. mollissima* hybrid), and a Connecticut Japanese-American-Chinese hybrid chestnut (Jaynes *et al.,* 1976). We then obtained permission from the Plant Protection and Quarantine Division of the USDA for the next step.

Our work then diversified: to the real world of sprout clumps of American chestnut trees in heavily wooded areas; to more work on the growth and behavior of our virulent and hypovirulent strains on synthetic media in the laboratory; and to more biochemical tests for dsRNA and a search for the presence of virus-like particles in our cultures of *E. parasitica.*

In most situations we can now cure a given canker on a tree and we are making progress in understanding the nature of hypovirulence. We know that:

1. Transmissible hypovirulence is a disease, or a group of diseases, of the fungus, producing reduced pathogenicity in the host, but not necessarily reduced vigor as a saprophyte;
2. It is controlled by genetic determinants in the cytoplasm;
3. These determinants are probably on, or associated with, dsRNA;
4. All hypovirulent strains examined so far by Peter Day and Allan Dodds contain dsRNA (Day *et al.,* 1977);
5. Allan Dodds has shown that in at least one strain the dsRNA is all encapsulated in virus-like particles, each surrounded by a lipid membrane (Dodds, 1979).

We now have hypovirulent strains from France, Italy, and North America, and American strains with hypovirulence derived from many of these sources. There is great variation in culture morphology and host pathogenicity among these strains, and this is one of our reasons for considering hypovirulence to be a group of diseases of the fungus with similar end results, as far as the tree is concerned (Elliston, 1979).

Our method of inoculating hypovirulent strains into cankers is to remove several (four to six) plugs of bark around the circumference of the canker and fill the hole with mycelium of one or several hypovirulent strains in Difco potato dextrose agar (PDA). The filled holes are then covered with masking tape (brown paper tape with an adhesive) to keep them from drying out (Puhalla and Anagnostakis, 1971). A field test with about 400 trees has been in progress since this past summer. The results of one season are very encouraging (Jaynes and Elliston, 1979).

Since we have had instances where a given hypovirulent strain would not cure a given canker, we started looking for a genetic system which would prevent successful hyphal anastomoses between strains, and therefore prevent transfer of hypovirulence determinants, which are all cytoplasmic. I have found and described (Anagnostakis, 1977) a system of vegetative incompatibility in *E. parasitica* controlled by at least six nuclear genes. Virulent strains, and normal strains isolated from hypovirulent strains can be paired in the laboratory on Difco PDA medium. If they are not compatible, the colonies will not merge and a ridge of asexual fruiting bodies (pycnidia) will form between them, along the barrage. Strains within any given group simply merge with each other on the agar and the hyphae anastomose. So far we have found 46 compatibility groups. In the host there is some evidence that a given hypovirulent strain will most easily cure a canker caused by a virulent strain in the same compatibility group, but that it can cure cankers caused by strains in some other compatibility groups as well, perhaps more slowly. Some of the field data of Jaynes and Elliston give more information on this, and controlled host tests with several hypovirulent strains and representative virulent strains from all of the compatibility groups are in progress.

In spite of the evidence from our Italian colleagues that blight is not presently a problem in Italy due to the natural spread of hypovirulence, we have seen no evidence that this biological control is spreading in our New England forest test plots. I have isolated ascospores from cankers cured with French-derived American hypovirulent strains and never found hypovirulent strains among the resulting clones. It may be that sexual reproduction somehow excludes the dsRNA or virus-like particles. Workers in the early 1900's in this country reached the conclusion that ascospores (which are airborne after discharge) are the primary source of new infections. J. Grente and T. Turchetti have told us that sexual reproduction is very rare on healed (hypovirulent) cankers in France and Italy. If hypovirulence cannot spread via ascospores, then a vector may be required. It is possible that some vector, such as a bird or an insect is responsible for the rapid spread of the curing strains in Italy, and that these vectors are not present here. We have an entomologist, Kenneth Welch, working with us now to consider this problem.

At the same time, Allan Dodds is working to purify and characterize *Endothia* viruses, and trying to find out if they are in fact associated with hypovirulence. Peter Day is continuing biochemical-genetics studies. John Elliston is studying the physiology and phenotypes of our hypovirulent

strains and doing field experiments on control and spread with Richard Jaynes. I am continuing my work on vegetative incompatibility and making controlled crosses of the fungus to gain more genetic information.

We hope that with this team effort we will make rapid progress in finding the best way to use hypovirulence for biological control in the United States.

LITERATURE CITED

Anagnostakis, S. L.
1977. VEGETATIVE INCOMPATIBILITY IN *Endothia parasitica.* Experimental Mycology 1: 306-316.

Anagnostakis, S. L. and R. A. Jaynes.
1973. CHESTNUT BLIGHT CONTROL: USE OF HYPOVIRULENT CULTURES. Plant Dis. Rep. 27:225-226.

Day, P. R., J. A. Dodds, J. E. Elliston, R. A. Jaynes, and S. L. Anagnostakis.
1977. DOUBLE-STRANDED RNA IN *Endothia parasitica.* Phytopathology 67:1393-1396.

Dodds, J. A.
1979. DOUBLE-STRANDED RNA AND VIRUS-LIKE PARTICLES IN *Endothia parasitica.* American Chestnut Symposium Proc., West Virginia University, Morgantown, WV.

Elliston, J. E.
1979. PATHOGENICITY AND SPORULATION OF NORMAL AND DISEASED STRAINS OF *ENDOTHIA PARASITICA* IN AMERICAN CHESTNUT. American Chestnut Symposium Proc., West Virginia University, Morgantown, WV.

Grente, J.
1965. LES FORMES HYPOVIRULENTES *D'Endothia parasitica* ET LES ESPOIRS DE LUTTE CONTRE LE CHANCRE DU CHATAIGNIER. C. R. Hebdomandaire Seances Acad. Agr. France 51:1033-1037.

Grente, J. and S. Sauret.
1969a. L'HYPOVIRULENCE EXCLUSIVE, PHENOMENE ORIGINAL EN PATHOLOGIE VEGETALE. C. R. Hebdomandaire Seances Acad. Sci. Serie D 268:2347-2350.

Grente, J. and S. Sauret.
1969b. L' 'HYPOVIRULENCE EXCLUSIVE" EST-ELLE CONTROLEE PAR DES DETERMINANTS CYTOPLASMIQUES? C. R. Hebdomadaire Seances Acad. Sci. Serie D 268: 3173-3176.

Jaynes, R. A., S. L. Anagnostakis, and N. K. Van Alfen.
1976. CHESTNUT RESEARCH AND BIOLOGICAL CONTROL OF THE CHESTNUT BLIGHT FUNGUS. In *Perspectives in Forest Entomology* (J. F. Anderson and H. K. Kaya, eds). Academic Press, New York.

Jaynes, R. A. and J. E. Elliston.
1979. CONTROL OF *ENDOTHIA PARASITICA* CANKERS ON AMERICAN CHESTNUT SPROUTS WITH HYPOVIRULENT STRAINS. American Chestnut Symposium Proc., West Virginia University, Morgantown, WV.

Puhalla, J. E. and S. L. Anagnostakis.
1971. GENETICS AND NUTRITIONAL REQUIREMENTS *OFENDOTHIA PARASITICA.* Phytopathology 61:169-173.

Van Alfen, N. K., R. A. Jaynes, S. L. Anagnostakis, and P. R. Day.
1975. CHESTNUT BLIGHT: BIOLOGICAL CONTROL BY TRANSMISSIBLE HYPOVIRULENCE IN *ENDOTHIA PARASITICA.* Science 189: 890-891.