VirTis 60 homogenizer at 48,000 rev/min for 1 hour while cooling the sample with ice water. Fragment length was determined from measurements of the T_m depression observed upon thermal dissociation of the fragments in 0.12*M* phosphate buffer (*8*). Reassociation buffer contained 50 percent formamide, 10 m*M* piperazine-*N*,*N*′-bis[2-ethanesulfonic acid] (PIPES), *p*H 6.7, and 1.0*M* NaCl. Our standard incubation temperature of 42°C was 21°C below the T_m of native, sheared DNA in this solvent.

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11. C_0t is defined as the concentration of DNA in moles of nucleotides per liter times time in seconds (*8*).
12. The genome size of *O. cinnamomea* is estimated to be 9.6×10^{10} nucleotides (*13*), or about 1.5×10^4 times as large as that for *Bacillus subtilis*. The DNA of *B. subtilis*, when reassociated under our conditions, has a $C_0t_{1/2}$ (C_0t required for 50 percent reassociation) of 2, and it can therefore be predicted that single-copy DNA sequences in the *O. cinnamomea* genome would be half reassociated at a C_0t of approximately 3×10^4.
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Chestnut Blight: Biological Control by Transmissible Hypovirulence in *Endothia parasitica*

Abstract. *Hypovirulence in* Endothia parasitica *is caused by a cytoplasmic determinant that is transferred by hyphal anastomosis in host tissue and in culture. Transmission of this determinant affects the virulence of the fungus to the extent that host invasion by previously virulent isolates is limited.*

Chestnut blight, caused by the fungus *Endothia parasitica* (Murr.) Anderson, eliminated the American chestnut as a dominant or codominant species throughout its natural range in North America. Sprouts of this tree have continued to grow from stumps, but before reaching maturity they are attacked, girdled, and killed by the pathogen. The virulent pathogen is still present, surviving and producing its abundant spores on the sprouts. The search for resistant American chestnut trees and attempts to produce resistant hybrids comparable to the original tree have not succeeded. The work reported here offers hope that the virulent strain of *E. parasitica* can be modified in nature so that the American chestnut can once again survive and multiply.

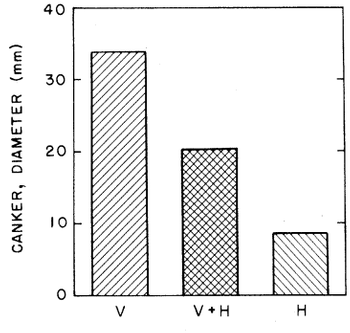

Fig. 1. Cankers formed by virulent (V) and hypovirulent (H) strains of *E. parasitica* inoculated as pairs, or singly, on field-grown American chestnut seedlings. There were 13 inoculations per tree (six V, one H, six V plus H) replicated once on each of six trees. Two French H and six American V isolates were used. The size of cankers was measured 54 days after inoculations.

A variant of *E. parasitica* low in virulence toward chestnut trees was first observed in Italy by Biraghi in stands of European chestnut (*Castanea sativa* Mill.) in which the disease was no longer spreading (*1*). Typical virulent forms of the fungus could not be isolated from these stands. The variant later found in France and in the Pyrenees was isolated and described as hypovirulent by Grente (*2*, *3*). The hypovirulent form (H) is not only weakly virulent itself but prevents more virulent forms (V) from vigorously invading the host tissue when both are together in the same canker (*2*, *3*). Grente (*4*) reported that H survives well in nature. When he artificially inoculated European chestnut plots, H appeared to dominate the *E. parasitica* population after 3 years. Grente and Sauret (*3*) speculated that hypovirulence was due to the presence of a transmissible determinant.

Anagnostakis and Jaynes (*5*) obtained cultures from Grente and demonstrated that H can limit the size of the canker formed by the French V strain on American chestnut trees. In their limited experiments, cankers formed by an American V strain were not restricted by using the French H strain.

We set out to find an H strain that could protect American chestnut trees from being killed by the American V strain, and to examine the nature of hypovirulence. We used hypovirulent strains 2025 and 2043 (a single spore isolate of 2025) from Grente and American virulent strains from infected native American chestnut trees growing in Connecticut. All isolates were grown on potato dextrose agar (PDA, Difco). Seedlings (3 to 8 years old) of American chestnut were used and inoculations, measurements, and isolations were as described (*6*).

Auxotrophic mutants of *E. parasitica* were obtained according to the procedures of Puhalla and Anagnostakis (*7*). Hypovirulent colonies of *E. parasitica* grown on PDA under lights have fewer pycnidia, less pigment, and a more milky appearance than virulent colonies. These cultural differences were expressed at all temperatures tested in the laboratory (10° to 30°C) and they correlated perfectly with virulence in the host (54 V and 56 H cultures were tested).

When trunks of American chestnut trees were inoculated with strains of American V and French H, alone and paired, cankers resulting from the combination were significantly smaller than those of V alone (Fig. 1). However, the mean size of the cankers from the paired inoculations was greater than that of H strains alone, indicating that protection was not complete. Isolations from the edges of the cankers formed by these paired inoculations yield-

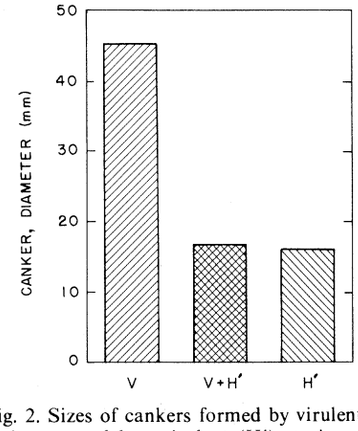

Fig. 2. Sizes of cankers formed by virulent (V) and recovered hypovirulent (H′) strains of *E. parasitica* inoculated as pairs, or singly, on field-grown American chestnut seedlings. There were nine inoculations per tree (three V, three H′, and three V plus H′) replicated once on each of eight trees. The H′ strains were isolated from cankers formed from V plus H paired inoculations. Size of cankers was measured 42 days after inoculations.

ed hypovirulent strains (H′) about half of the time. When H′ strains were used in paired inoculations with V, there was no significant difference between the mean canker size of the paired H′ and V inoculations and that of the H′ alone (Fig. 2). While H′ alone in this experiment produced a larger canker than H alone in the previous experiment, the growth of V was also greater, an indication that the second experiment was conducted under conditions more favorable for pathogen growth.

Two months after the trees were inoculated, isolations were made from five points equally spaced around the circumference of the cankers, transferred to PDA, and scored for morphology in culture as V or H (H and H′ have the same morphology). Of 40 strains isolated from H plus V inoculations, 18 had virulent morphology, 20 hypovirulent, and 2 sectored; whereas of 43 strains isolated from H plus V inoculations, 5 had virulent morphology and 38 were hypovirulent. We performed heterokaryon tests (*8*), using auxotrophic markers to establish whether or not hypovirulence was cytoplasmically determined. An American virulent methionine auxotroph (*met*-1) (*7*), and a lysine auxotroph (*lys*-1), induced by ultraviolet irradiation and derived from an H′ hypovirulent isolate, were inoculated singly and as pairs in the trunks of two American chestnut seedlings. There were two replications of the inoculations on each tree. After 90 days, isolates were taken from the edges of the resulting cankers. Of the four paired inoculations, canker size was restricted in two and only methionine auxotrophs were isolated from them. The lysine auxotroph could not be reisolated from either single or paired inoculations. Methionine-requiring isolates from restricted cankers were then inoculated into trunks of chestnut seedlings, and the sizes of resulting cankers were compared with those from virulent *met*-1. The cankers formed by recovered methionine-requiring isolates had a mean diameter of 12.5 mm, and those formed by the virulent *met*-1 strain were 38.1 mm in diameter, thus the recovered *met*-1 isolates were hypovirulent. Heterokaryons were forced in culture with the use of a *met*-1 H′ strain and an arginine auxotroph (*arg*-1) derived from an American virulent strain (*7*). Methionine- and arginine-requiring clones were recovered from uninucleate conidia produced by the heterokaryon. Clones of both types had the morphology associated with hypovirulence.

Since hypovirulence appears to be the result of a cytoplasmic determinant transmitted from hypovirulent strains to virulent ones by hyphal anastomosis, the relative abilities of H′ and H strains to limit the size of a canker when coinoculated with a V strain could be due to heterokaryon incompatibility between the French H strain and the American V strain. The H′ was probably derived from American V and therefore heterokaryon incompatibility with the American V was no longer a factor preventing transfer of the cytoplasmic determinant. Furthermore, isolation from the cankers of the paired inoculations of H′ plus V yielded *E. parasitica* with hypovirulent morphology much more frequently than from the cankers of H plus V paired inoculations, which is evidence that hypovirulence is transmitted to American V strains more readily by H′ than by H.

Since in the heterokaryon transfer experiments, the previously virulent strains were reisolated and shown to be hypovirulent, limitation of canker size by hypovirulent strains is not due to elimination of the virulent strains. Induction of a localized host defense response by the H strain as an explanation for hypovirulence has not been ruled out, but, if it is involved, the genes responsible for causing this induction are carried by the cytoplasmic determinant.

Our results in the laboratory, greenhouse, and field, plus the original observation in Europe that hypovirulence can control chestnut blight in nature, suggest that this fungal strain may become a control for the disease in the United States.

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Flagellar Coordination in *Chlamydomonas reinhardtii*: Isolation and Reactivation of the Flagellar Apparatus

Abstract. *The flagellar apparatus (both flagella with basal bodies and accessory structures) of* Chlamydomonas reinhardtii *was isolated from a wall-less mutant and induced to swim in the presence of adenosine triphosphate. The movement of the flagella of the isolated apparatus was largely synchronous and indistinguishable from the movement of flagella on living cells. These observations suggest that the mechanism of flagellar coordination in this biflagellate alga is largely intrinsic to the structure of the flagellar apparatus itself.*

The unicellular, biflagellate alga *Chlamydomonas reinhardtii* offers a simple model system in which to study the mechanism of flagellar coordination. *Chlamydomonas* swims by means of two anteriorly located flagella which generate a ciliary type of beat with two distinct phases: an effective stroke in which the two flagella are held rigid and swept simultaneously back past the cell, bending only near their bases, and a recovery stroke in which a wave propagated from the proximal end of each flagellum passes out to the distal tip (*1*). Electron microscopy of thin sections through the anterior region of *C. reinhardtii* shows that the flagella arise from two basal bodies which lie at approximately a 90° angle to each other (in a "V" configuration) and are connected at their proximal ends by two striated fibers and at their midlateral faces by a single striated fiber (*1*). We refer to the collection of the two flagella and the basal bodies with connecting fibers as the flagellar apparatus.

Demembranated individual flagella from *C. reinhardtii*, detached from their basal bodies, can be reactivated in vitro (*2*). We now report a procedure for the isolation of the entire flagellar apparatus from *C. reinhardtii* under conditions that allow its subsequent reactivation and analysis in vitro.

A wall-less mutant of *C. reinhardtii*, strain CW92 (*3*), was used to facilitate isolation of the flagellar apparatus. Cultures (1.5 liters) of CW92 were grown to approximately 10^6 cells per milliliter in medium 1 of Sager and Granick (*4*) supplemented with 0.2 percent sodium acetate; they were grown at 25°C with continuous aeration and on an illumination cycle of 14 hours light and 10 hours dark. The cells