

SUMMARY OF CHESTNUT RESEARCH AT DUKE UNIVERSITY
1980 TO 1981

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ABSTRACT.--Research on the potential for transmission of hypovirulence in Endothia parasitica to chestnut Castanea and oak Quercus in North Carolina is summarized and updated since the last U. S. Forest Service American chestnut cooperators' meeting held January 1980 at Pipestem, West Virginia. Plans for future directions in this research are discussed.

By way of introduction and since two major studies in our program will be presented as separate papers, we will mention these first but only in terms of their status for the future. All other subprojects will follow by topic, summary of work accomplished, and future plans.

Incidence of *Endothia parasitica* in North Carolina

This 33-county survey of the mountain and Piedmont regions of the state is now complete, including vegetative compatibility (v-c) typing of 410 *E. parasitica* isolates thus derived, and will be reported later by Bruce Nash. This phase of our work is now terminated and no continuation along these lines is planned.

Detection and Evaluation of Survival in American Chestnut

Preliminary data from a four-tree sample, to date, on this project will be presented separately in these proceedings by this senior author.

Our plans, which are detailed in the paper, can now be implemented with the appointment of a research assistant this January 1982. The immediate winter schedule calls for: 1) mail survey to mill owners, buyers, and logging contractors in the mountain and foothill counties of North Carolina to enlist their help in locating large (21.6 cm d.b.h.) American chestnuts, *Castanea dentata*, for hypovirulence/resistance evaluation; 2) collection and storage of scionwood from all study trees for spring grafting attempts to Chinese chestnut, *C. mollissima*, rootstock; and 3) completion of isolate screening

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from study trees to detect within-tree v-c patterns, abnormal cultures, and conversion ability of appropriate pairings. Next spring, pathogenicity trials with all suspect hypovirulent (H) isolates from the study trees, or a representative sample will be compared with virulent (V) isolates via inoculation of American chestnut sprout stems in Watauga County provided for our use by the Johnson Lumber Company of North Wilkesboro. The remainder of the field season will concentrate on intensive sampling of and isolate processing from additional study trees located from our mail survey and associated infected hosts found within 152 m of each study tree. Four candidate trees (d.b.h. range, 29.0 to 82.3 cm) have already been located for possible study selection in 1982.

Epidemiology of *E. parasitica* on the Duke Forest

Epidemiological monitoring of *E. parasitica* on the Duke Forest is underway and is focused upon a \pm 50-year-old, 3.8 ha Asiatic chestnut *C. crenata* and *C. mollissima* plantation, representing an established epidemic, and two, ca 0.4 ha plantings of 2-0 Chinese chestnut stock planted in the spring of 1980 and 1981, representing potential for epidemic development. All three plantations have been stem mapped and canker incidence data is now being collected from the older one while new infections in the younger ones will be monitored by means of annual examination. Isolates will be obtained from all infected trees, all new infections, and from the older plantation, all cankers per tree in at least 10 percent of the multiple-cankered stems. All isolates will be tested for intra- and inter-tree v-c type frequency and distribution; culturally abnormal isolates will be processed for hypovirulence characteristics.

Beyond each plantation perimeter, all oak hosts *Quercus alba*, *Q. coccinea*, and *Q. stellata* to a distance of 305 m will be located and sampled for *E. parasitica* isolate yield and v-c/possible H-strain characterization.

A student project (Leininger 1981) which involved sketch mapping of and collection of incidence data from 220 native oaks in a 32 ha stand adjacent to the oldest Asiatic chestnut plantation, showed infection of white, post, and scarlet oak, on an isolation basis, at levels of 1.8, 21.4, and 37.5 percent, respectively. Isolates from three white, 18 post, and 24 scarlet oaks were grown in opposition culture, according to the methods of Anagnostakis (1978), with yields of six definite and seven possible v-c groups. Two v-c types, in particular, were consistent within group infections of five and 11 trees, primarily composed of scarlet oak.

More recently, all of these isolates have been grown in 9 cm potato dextrose agar (PDA) plates through three successive transfers; two from post oak and four from scarlet oak, all of which appeared normal initially, are now showing decided growth reduction and irregular colony margins. These will be tested further for conversion ability, using the methods of Anagnostakis and Day (1979), and pathogenicity, while the tree sources will be sampled intensively for additional isolate yield and characterization.

Cross-Inoculation Study

Ten randomly-chosen isolates of *E. parasitica* isolated from two sources each of American chestnut, Chinese chestnut, post, scarlet, and white oaks were

cross-inoculated on May 23 to 28, 1980, and replicated again on June 24 to 26, 1981, into each host represented. Each of the 10 isolates, plus a control, was replicated on at least 10 individuals of each host (total of 1100 inoculations). Inoculations of American chestnut were done at the Coweeta Hydrological Laboratory Forest on different dates as travel would permit.

To date, only the chestnut species inoculations have been sampled and rated, primarily to avoid canker coalescence, whereas the very slow canker development in the oaks has postponed canker evaluation until at least the 1982 field season. It is evident, however, that isolates derived from the oak species are just as virulent on the chestnut species as isolates from chestnut.

Applied Hypovirulence in Endothia-Infected Live Oak

This student project (Blair 1981) showed that 11 of 16 virulent isolates of *E. parasitica* from live oaks, *Quercus virginiana*, at Fort Monroe, Virginia were converted to hypovirulent forms when placed in opposition culture with a slurry of seven known hypovirulent strains. Three weeks after treatment (July 2, 1981) of portions of cankers on the isolate-source oaks with ten replicates each of a slurry and spray formulation or the seven hypovirulent strains, hypovirulent strains were reisolated from all four slurry-treated cankers sampled, one of which also yielded normal isolates, but only from two of four spray-treated cankers. The treated cankers will be examined in July 1982 for canker change, if measurable, and isolate yield from all treatments will be evaluated.

Blair (1981) noted that two of the isolates from live oak behaved in plate culture like H-strain isolates. Since then, we have grown 15 of the live oak isolates, including the two in question, in 9 cm PDA plates through four successive transfers and have found that the two isolates and one more show cultural abnormality typical of H-strain isolates. These isolates will be tested for pathogenicity in American chestnut and the tree sources will be scrutinized for possible symptom correlations along with sampling for additional isolate yield characterization.

Insect Vector Studies

Studies of ants in association with cankered and uninfected scarlet oak and Chinese chestnut on the Duke Forest are being continued by Tim Albaugh for his MF project requirements. Development of an agar medium for selective isolation of *E. parasitica* from suspect ants compared chestnut bark, tannic acid (10 and 50 ppm), and PDA. Tannic acid at 0.5 percent gave best results and utility, being exceeded only by PDA in growth response of the fungus (5.9 versus 4.3 mm/day). Only tannic acid, however, showed selectivity when the different media were inoculated with a contaminated conidial suspension of *E. parasitica* in dilution plate assay.

At various times during July to August and October 1981, especially as weather conditions varied, ants were collected from cankered and uninfected scarlet oak and Chinese chestnut trees and plated as 1 ml dilution washes on 0.5 percent tannic acid agar. To date, the fungus has been isolated in greatest frequency and inoculum yields from *Aphaenogaster lamellidens* up to 2 days

following rain. Only the collections from infected scarlet oak in June to July were positive; stem washes from all ant-source trees also yielded the fungus on tannic acid agar.

Presently, mode of transmission is being studied by caging ants infested with *E. parasitica* in fresh wounds on intact trees and excised, end-sealed stem sections of scarlet oak and Chinese chestnut. In addition, by exposing ants to H-strain inoculum, attempts will be made to obtain conversion of compatible V-strains on Chinese chestnut by field caging experiments. Screening of host-associated ant populations for quantification of vectoring potential will be continued.

Another MF project study by Susan O'Bryan involves the field exposure of artificially-inoculated V- and H-strain cankers in excised oak and chestnut stem sections for seasonal monitoring of insect attraction. Insect species collected from these cankers in sufficient numbers will be divided into separate lots for reference and identification, screening for isolate yield, and wound-caging on Chinese chestnut. Only the pilot-testing phase of this study has been developed to date.

Etiology of *Endothia gyrosa* on Branch-Pruned Willow Oak

In this MF project study by Greenberg (1981), an 86-tree sample of pruned willow oak, *Q. pheZZos*, in Durham, North Carolina showed canker/dieback incidence of 42 percent. On average, infections involved less than four branches per tree at stem origin heights less than 7.6 m and occurred most frequently on smaller trees (25 to 64 d.b.h.; range to 102 cm).

Inoculations with *E. gyrosa* on freshly-pruned willow oak branch stubs on August 13, 1980 averaged 6.6 cm penetration with 7 of 10 showing fruiting of the fungus by mid-December, thus confirming a new host record. No additional work is planned.

Literature Cited

- Anagnostakis, Sandra L. Testing *Endothia parasitica* strains for vegetative incompatibility. MacDonald, William L.; Cech, Franklin C.; Luchok, John; Smith, Clay, eds. Proceedings of the American chestnut symposium; 1978 January 4-5; Morgantown, WV. Morgantown: West Virginia University Books; 1978:101-102.
- Anagnostakis, Sandra L.; Day, Peter R. Hypovirulence conversion in *Endothia parasitica*. *Phytopathology* 69:1226-1229; 1979.
- Blair, Gem. Biocontrol potential of applied hypovirulence in *Endothia*-infected live oaks at Fort Monroe, Virginia. Durham, NC: Duke University; 1981. 23 p. MF Project Report.
- Greenberg, J. Dieback potential of *Endothia gyrosa* on branch-pruned willow oak. Durham, NC: Duke University; 1981. 18 p. MF Project Report.

Leininger, Ted. Vegetative compatibility types of *Endothia parasitica* in scarlet, post, and white oaks on Compartment 70 of the Duke Forest. Durham, NC: Duke University; 1981. 20 p. MF Project Report.

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