CHESTNUT BLIGHT--PRIORITIES FOR RESEARCH

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ABSTRACT. --This p*aper is an attempt to sharpen the original objectives and assign priorities for American chestnut research programs. A group discussion session was used to try to identify gaps in existing knowledge inhibiting American chestnut research progress, identify areas where research progress would indicate a high probability of success and where future research efforts should be directed. During the time frame of the meeting, it was not possible to obtain a specific concensus of research priorities. Solutions in one research area are required to solve problems in others. Priorities ultimately will be determined by the review process given to grant funded proposals.*

Introduction

Periodic assessment of progress toward objectives coupled with critical reevaluation of the original goals are necessary elements in all research programs. Often these steps lead to a sharpening or refocusing of the original research objectives--sometimes to a reordering of priorities.

The final discussion session of this meeting was devoted to such an examination, Its purpose was to identify (1) gaps in knowledge that are blocking research progress, (2) areas where research progress indicates a high probability of success with added effort, and (3) where future research efforts should be focused.

A schematic chronology of chestnut blight (Figure 1) was used to focus discussions on critical areas of the disease cycle that either influence or are influenced by the phenonemon of hypovirulence.

The original objective of the current chestnut blight research program was to shift, by means of hypovirulence, the outcome of infection from death to survival, that is, to increase the relative numbers of trees in categories (6), (8), and (10), as shown in Figure 1.

The following section presents the items discussed under each topic (numbers correspond to Figure 1), pertinent points that emerged in the discussions, and a statement of the topic's relative priority in future chestnut blight research on hypovirulence.

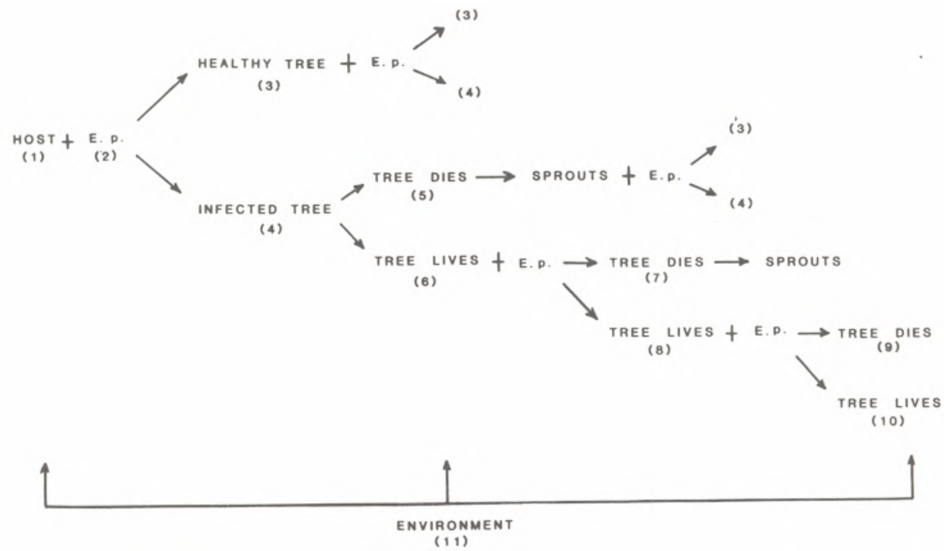

Figure 1. This scheme indicates that healthy host trees (1) exposed to the pathogen *Endothia parasitica* (E. p.) (2) either do not become infected (3) or do become infected (4). Infected trees develop cankers that either kill them (5) or do not kill them (6). The disease may repeat on sprouts from stumps of killed trees. Cankered trees that survive (6), either because they are genetically resistant or because the fungus is less virulent, are exposed repeatedly to reinfection, and either succumb (7), (9), and so on or survive (8), (10), and so on. Influencing all of these agents and their interactions are factors of the physical environment (11).

Topic (1) Factors that influence the relative susceptibility of host trees

a. Host genetics
b. Tree status (size, age, origin)
c. Ecological forest relations (density, distribution patterns, geography)
d. Non-American chestnut hosts (oaks, Chinese chestnut)

a. Evidently American chestnut is not resistant to virulent strains of *E. parasitica.*

- Because past efforts to find trees resistant to normal strains of *E. parasitica* have been unsuccessful, a major effort to locate trees that are resistant to hypovirulent (HV) strains probably is not warranted. However, researchers comparing host responses to HV isolates should distinguish between the effects of fungi debilitated to varying degrees, and effects attributable to different host genotypes.

Previous efforts in tree genetics focused on either discovering resistant American chestnut trees or incorporating resistance factors from other species into American chestnut genomes. With HV strains, the focus is shifted to discovering diseased trees with cankers that are superficial and persistent and that produce viable propagules of HV strains.

- With this shift in focus, host genotype may become more important. Researchers should recognize that it may now be possible to select desirable trees from within genomes previously considered undesirable.

b. The influences of such tree variables as size, age, and origin (sprout versus seedling) on relative susceptibility were alluded to in several papers presented at this meeting.

- The possible influence of such variables must be recognized and well documented because differences in host/pathogen interactions involving HV may reflect more critically, the differences in tree condition.

c. The numbers of chestnut trees per unit area, their distributions relative to each other (degree of aggregation or isolation--of individuals or stands), and the geographic locations (growing within or outside of the natural range) are variables that seem to influence the probability of infection.

- Dense stands or local aggregations of chestnut were previously considered undesirable because they favored spread, development, and persistence of the virulent pathogen. Such relationships may need to be reevaluated in light of HV. It may become necessary to manipulate these variables to ensure the spread and development of less competitive HV strains.

American chestnuts are growing in Michigan beyond the natural range of the species. In many former groves, trees were severely blighted--many were killed. But, in Michigan, in contrast to forests in Appalachia, some large trees and many of the sprouts from root systems of killed trees did not succumb to infection. Cankers from such survivors have often yielded HV isolates.

- The situation in Michigan provides the only clear parallel in this country to that reported in Europe. It is a dramatic natural demonstration that biological control, presumably HV, can arrest the spread and development of chestnut blight in American chestnut. It is very important to understand what is happening in the Michigan "Laboratory" with respect to the source of HV, mechanisms of transmittance, and the patterns of spread and development of HV over time within and between trees and stands. It may comprise the model needed to clarify the ecological relationships affecting the use of HV.

d. The significance of non-chestnut hosts--especially scarlet and post oaks--in the disease cycle and especially in relationship to HV is not clear. It is not known if oak cankers can be converted to HV types--and if so, whether they will continue to persist and serve as sources of HV inoculum.

- The probable role of oak-inhabiting insects (especially ants) as carriers, the basal location of many oak cankers, their persistent non-lethal nature, and their abundance in certain areas warrant a continued research effort to clarify the significance of oak hosts to HV.

Conclusion: Intermediate priority

- Except for studies on non-chestnut hosts-- and on understanding the situation in Michigan (see Topic 2 as well)-- the above items that deal in general ways with host tree susceptibility are somewhat lower in priority than those that relate to the pathogen itself (Topic 2) or to specific aspects of the host-pathogen interaction (Topics 4, 6).

Topic (2) Factors that influence epidemiology of HV *Endothia parasitica*

A. Field Level

a. Inoculum density
b. Inoculum type (propagules)
c. Means of dissemination
d. Infection courts
e. Seasonal effects
f. Ecological niche (saprophyte - parasite)
g. Vegetative compatibility (v-c)

B. Molecular Level

h. Molecular biology of hypovirulence
i. ds-RNA
- -its identification and characterization
- -its form in host
- -its means of transmission
- -its origin

2A: a-g. The basics to understanding HV epidemiology are: knowledge of how much inoculum is required to initiate HV infections in nature; what propagules are responsible and how they differ from virulent types; how these propagules are disseminated over short and long distances, to what infection courts, and at what time of year; in what niche HV is perpetuated; and what inter-strain factors regulate conversion. Such information is also requisite to the design of control programs utilizing HV.

Discussions of the factors influencing epidemiology of HV revealed that much of this information is either lacking or not readily available--not only for HV, but also for normal strains as well.

- We need to learn what is known about the epidemiology of normal strains so valid comparisons can be made to HV strains.

- It would be valuable to screen the old literature for pertinent information that, when reinterpreted in view of today's situation, may yield important clues. Often, vital information obtained by astute observers is hidden in out-of-print or obscure literature.

Basic epidemiological questions are difficult to answer--and often entail studies that are both money and labor intensive.

- A new approach may be necessary. We may need to reinstate the practice of patient observation utilized by our predecessors. Patience is also the key to demonstrating the efficacy of HV in the field. Because of the normal lag effect that always occurs in the development of disease epidemics, it will require time before an HV "epidemic" can be recognized or reach the stage of measurement.

The development and spread of HV probably will occur everywhere eventually, but recognition of these phenomena may be very difficult in areas where endemic "background counts" of virulent normal strains are innundative.

- The dynamic situation in Michigan may provide a special opportunity to "sit and watch". Good observations often result from seizing situations--sometimes fleeting--that are unique in time and place. The stage of disease development and the unusual HV and v-c relationships in Michigan make epidemiological studies there of high priority.

2B: h-i. How important is it that we understand HV at the molecular level? Do we need to know the nature of ds-RNA, how it occurs in its fungal hosts, how it is moved from one fungus to another--from one location to another, where it comes from?

- The phenomenon of HV does exist--its effects have been demonstrated both naturally (isolated individual diseased surviving trees in Appalachia; reduced tree mortality in Michigan) and artificially (conversion and 'control' of individual inoculated cankers in many locations and lowered mortality rates following massive dispersal of inocula in Connecticut. And, evidence of spread of HV following its artificial introduction is just beginning to accumulate. All of this has occurred with very little understanding of HV at the molecular level. Yet, until the questions posed above are answered, future approaches to using HV will remain strictly trial and error.

Information at the molecular level may be acquired relatively rapid because of the recent advances in techniques in these fields.

- Many new techniques have yet to be applied to this problem (for example, gene recombinant studies to reduce or interfere with virulence). Molecular level information, once acquired, must be placed in the context of natural systems through concommitent studies in field epidemiology. Integrating information from research at all levels will help to ensure that control programs utilize the right materials, at the right time, at the right place, in the right way.

<u>Conclusions: High priority</u>

- Research on factors affecting the epidemiology of HV strains of *Endothia parasitica is* a high priority. It is important to "relearn" what is already known about the virulent strain and to compare that to results from new studies on HV isolates. An understanding of the molecular biology of HV is of great importance to the intelligent development of effective methods to manipulate or manage HV at field levels. A multi-level research thrust will produce more results faster than will research at only one level.

Topic (3) Factors that either contribute to the occurrence of or relate to disease-free trees (survivors).

a. Resistance mechanisms
b. Escape mechanisms
c. Methods to vegetatively propagate healthy survivors (also desirable trees in other categories: hybrids for testing, trees with "desirable" cankers, and so on.)

a-b. It is unlikely that resistance or escape mechanisms will figure prominantly in research programs on HV--especially where abundant virulent inocula exist.

- Researchers challenging trees with debilitated isolates should be alert, however, to the possible influence of host genetics (see Topic 1).

c. Research has now demonstrated that American chestnut can be propagated in *vitro.* Continued research is needed to refine and improve these techniques

- Clonal "testers" are needed for critical evaluation of specific HV factors; American x European hybrids should be multiplied to assess their responses to HV in different areas; and, because some surviving trees in Michigan have better form and quality than expected, it may become important to propagate and test them under a broader range of conditions.

<u>Conclusions: Low to intermediate priority</u>

- Except for efforts on vegetative propagation--which have applicability elsewhere as well--research on this topic is a relatively low priority.

Topic (6, 8, 10, and so on) Factors responsible for the survival of cankered trees

a. Host-parasite interaction: bark cankers
b. Factors influencing host responses
c. Desirable host responses

a. When infection by *E. parasitica* is successful, the host-parasite interaction results in formation of bark cankers. Whether infected trees die (5) or survive (6, 8, 10,...) depends on their individual responses to infections by particular parasites.

- Presumably, most non-lethal cankers reflect host responses to HV strains of *E. parasitica.* Non-lethal cankers differ in appearance and consequence. Some are deep but limited laterally, some are superficial but extensive and persistent, some are combinations. Some result in little apparent adverse effect on tree form and growth-- some markedly affect the growth habit and shape of the tree.

b. Variations in canker appearances and effects probably reflect variations in such host responses as callus formation and necrophyllatic periderm establishment.

- Such variable host responses may result from differences in: strains of *E. parasitica* ("factors" of HV, degree of debilitation); timing of infection (seasons, tissue phenologies); tissues infected (age, infection courts); interval between infection and response (time before tree "recognizes" infection); and tree condition (energy status).

c. The host responses (type of cankers) incited by HV strains versus those incited by virulent strains are "the bottom line" of this research program.

- Desired are cankers that (1) are superficial (do not kill cambium and seriously maim trees), (2) produce viable spores carrying HV, that have sufficient diversity in v-c to effect conversion of virulent cankers in the region of concern, and (3) bear these spores for long periods of time (comprise persistent reservoirs).

A search for trees with "desirable" disease characteristics is quite a different approach from that usually taken in forest pathology, but one which has parallels in other biological control systems (take-all decline of wheat, gypsy moth control by parasites and diseases).

- The development and maintenance in the forest ecosystem, via HV, of mature chestnut trees, even though they are misshapen and of poor timber value, is a desirable first step. Such trees would be the means for genetic interchange and serve as a source of valuable wildlife mast.

Conclusions: High priority

- This area of research--the host response--is considered a high priority. It is very important to understand why a particular tree responds differently to infection by different HV isolates, or why different trees respond differently to infection by specific isolates.

Summary

- It was not possible to obtain a "finely tuned" concensus of research priorities. Factors influencing the trees, the pathogens and their interactions are so intimately associated that research on each area is important for success in the HV program. Solutions in one research area are required to solve problems in others.

Priorities ultimately will be determined by the internal and external reviews given to grant proposals. Sources of funds for grants (other than USDA, Forest Service) should be explored--especially for proposals that are well prepared and scientifically competitive.