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PROSPECTS FOR CONTROL OF DUTCH ELM DISEASE - BIOLOGICAL CONSIDERATIONS

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Abstract. Dutch elm disease continues to rank as a tree disease of enormous importance. The present report surveys a number of biological approaches which give some measure of control for the fungal pathogen and its beetle vectors. While procedures such as the use of pheromones to trap beetles and the sanitation of dead and dying trees are assessed, newer studies are discussed, such as the search for disease-causing agents in the fungal pathogen and the application of biotechnology to the disease. We review such concepts as an understanding of the pathogenicity genes of the fungus, the transformation of microbes normally associated with beetle vectors so as to become pathogenic to these insects, and the use of biotechnological experiments concerned with the elm.

In spite of the numerous studies directed at control of Dutch elm disease, (DED), this disease continues to cause serious losses of elms. Apart from the incalculable esthetic loss, the estimated cost of removing dead and dying elms in the United states is $100 million per year. This figure does not include costs for tree replacement, disease management, or property depreciation. Worldwide, elms are worth billions of dollars (Campana and Stipes, 1981).

Research on DED has emphasized an integrated approach whereby a number of proven techniques are used to manage the three primary components of DED—the host tree, the beetle vector, and the causal pathogen. In recent years, certain research has focused on biological agents that could be used to control or manage the beetle vector and fungal pathogen. Genetic engineering is another new approach that can be applied to the three components of DED. This paper considers several biological approaches that have been used against DED; some of them have been successful in laboratory programs but have not been effective in the field. In addition, the paper summarizes potential techniques that may be effective to all three components of the disease: the beetle vectors, the pathogen, and the host.

Beetle Vectors

Nematodes. The DD-136 strain of the nematode, *Neoaplectana carpocapsae,* has a special potential for elm bark beetle control in that it infects all three major beetle vectors of DED: *Scolytus scolytus* (Finney and Mordue 1976; Finney and Walker 1977), *S. multistriatus* (Poinar and Deschamps 1981), and *Hylurogopinus rufipes (Tomalak and Welch 1982).* Finney and Walker (1977) sprayed elm logs infested with *S. scolytus* with the DD-136 strain and found that the nematodes were efficiently dispersed, entering the logs through entrance holes in the bark. A significantly higher population of dead, nemotode-infected insects was found compared to the controls.

In laboratory studies, Poinar and Deschamps (1981) observed that infection of *S. multistriatus* with *N. carpocapsae* and with the nematode, *Heterorhabditis bacteriophora,* resulted in the death of larvae, pupae, and adults. Moreover, the nematodes multiplied inside all host stages and released juveniles that were infective to other hosts.

In other laboratory studies, Tomalak and Welch (1982) noted that larval stages of *H. rufipes* were more susceptible to the DD-136 strain of *N. carpocapsae* than pupae and adults; however, beetles at all stages, including eggs, died within 1-7 days at dosages as low as 15 nematodes per beetle. In a field trial, beetle emergence in moist,

nematode-treated, upright bolts of elm was reduced by 80%. Tomalak and Welch noted that the main problem with the use of nematodes is that they are not sufficiently resistant to desiccation. The nematodes must gain entrance to bark beetle galleries through the entrance holes. With moist bark this does not appear to present any difficulty to the nematodes; with dry bark, few nematodes can gain entrance.

Fungi. Fungi used as antagonists of elm bark beetles are, like nematodes, dependent upon a favorable environment. In the laboratory, Doberski (1981a) observed that isolates of *Beauvaria bassiana, Metarhizum anisopliae,* and *Paecilomyces farinosus* were pathogenic on *S. scolytus* larvae. The most and least virulent isolates were, respectively, isolates of *B. bassiana* and *M. anisopliae*; isolates of *P. farinosus* were moderately virulent. Adult bark beetles also were susceptible to infection by isolates of *B. bassiana* and *M. anisopliae.* Doberski (1981b) noted that one strain of *B. bassiana* and one of *P. farinosus* caused infection of beetle larvae, even at 2°C. With two strains of *M. anisopliae,* no infection of larvae was observed at temperatures below 10°C. Infection of adult beetles by *M. anisopliae* and *B. bassiana* occurred at temperatures of 15°C and 20°C and with *B. bassiana* at 25°C. At 25°C, beetles tended to succumb to bacteria. *B. bassiana* and *M. anisopliae* caused some infection at relative humidities from 51-100%. Infection by *P. farinosus* ceased at relative humidities at or below 74%.

Fungi naturally associated with the bark of elms may play a significant role in colonization of elms by bark beetles. In the north and west of Britain, the fungus *Phomopsis oblonga* was observed as the principal colonizer of the bark of wych elms (*U. glabra*) and smooth-leaved elms (*U. carpinifolia)* after their death through DED (Gibbs and Smith 1978, Webber (1981a,b) reported that *P. oblonga* prevented successful breeding or colony development of scolytid beetles in wych elms. In the laboratory, when beetles were given a choice to breed in logs with or without *P. oblonga* infestation, over twice as many galleries were produced in the unifested logs. In experiments offering no choice, the mean number of galleries was not significantly different in *P. oblonga*-infested logs and in logs free of this fungus. The mechanism by which *P. oblonga* inhibits colonization by *Scolytus* is not known. Weber (1981b) suggests that *P. oblonga* may produce metabolites that render elm bark unpalatable or even toxic to beetle larvae. The fact that *P. oblonga* reduces the moisture content of bark may contribute to beetle rejection of the bark as breeding material. Finally, it appears that *P. oblonga* may cause a decrease in nutrients necessary for development of beetle larvae. *P. oblonga*-infested bark contains less amino acids, simple sugars, and starch than unifested bark, and this reduction in nutrients may be a cause of the failure in beetle breeding or development of larvae.

Aggregation attractants. *S. multistriatus* adults are attracted to declining elms in which they breed by primary, host-produced odors (Meyer and Norris 1967) and by beetle-produced pheromones (Peacock et al. 1971; Pearce et al. 1975). Studies since 1974 have been aimed at determining whether mass trapping that incorporates the three main components of the *S. multistriatus* aggregation attractant— 4-methyl-3-heptanol, alpha-multistriatin, and alpha-cubebene — can be effective in DED control programs.

Mass trapping has been evaluated for preventing beetle immigration into isolated groves of DED-free elms (Lanier 1978, 1979, 1981, 1982), for reducing beetle populations and DED in large citywide programs (Cuthbert and Peacock 1979; Peacock 1982), and for suppressing beetle populations in small, isolated towns in eastern California that have large beetle populations but are free of DED (Birch 1979; Birch et al. 1977; Birch et al. 1981). Attractant lures have been used to bait elms that were killed by cacodylic acid in trap-tree programs (Lanier 1981, 1982; O'Callaghan et al. 1980).

Results of mass trapping programs have been mixed. It appears that trapping has reduced DED levels where traps were deployed around small groves or scattered stands of elms (Lanier 1978, 1979, 1981, 1982). But there is no indication that trapping had a significant, measurable effect on beetle populations or disease levels in large citywide programs (Cuthbert and Peacock 1979; Peacock et al. 1981; Peacock 1982), nor is there evidence that trapping affected beetle

populations in isolated, disease-free stands of elms in California studies (Birch 1979; Birch et al. 1977, 1981). The reasons for the discrepancy in these results were discussed by Peacock (1982) and Lanier (1982).

Recent evidence (Peacock 1982; Peacock et al. 1984) indicates that the activity of commerical attractant lures used in *S. multistriatus* mass trapping can be enhanced by additional chemical compounds, particularly with unidentified volatiles found in elm tissue. It must be determined whether these compounds can be isolated and identified, and whether a more powerful lure containing these compounds would significantly improve mass-trapping programs.

H. rufipes apparently does not produce powerful, long-range pheromones, but it does appear to respond to elm volatiles (Lanier 1982). These host compounds may be sufficient to account for the successful colonization of suitable brood wood by this beetle. Studies to identify the attractive volatiles are in progress. Obviously, a lure containing attractants for both North American vectors of DED would significantly improve trapping programs.

Aggregation attractants are used by *S. scolytus* in locating breeding sites. In contrast to *S. multistriatus,* the females of which produce pheromones, the aggregation pheromones of *S. scolytus* ((-)-isomers of threo and erythro 4-methyl-3-heptanol) are produced when unmated male beetles bore into elm (Blight et al. 1978; Blight et al. 1979); alpha-multistriatin, which is produced by both male and female *S. scolytus* (only in trace amounts by males), apparently does not influence the behavior of this species (Blight 1982).

Three compounds from elm, alpha-cubebene, (-)-alpha-pinene, and (-)-limonene, have been evaluated as attractants and pheromone synergists for *S. scolytus.* Results with cubebene have been varied, but a mixture containing low concentrations of this compound and 4-methyl-3-heptanol apparently is more attractive in the field than the latter compound alone (Blight 1982).

Further work on pinene is justified because it seems to enhance the activity of 4-methyl-3-heptanol, making it a potential substitute for the more costly and less available alpha-cubebene. There have been no large-scale field studies to evaluate aggregation-attractant systems for use in *S. scolytus* control programs, but research is in progress.

Sanitation. The removal and destruction of infested or potential beetle breeding material have continually been emphasized for DED management. However, beetle control through sanitation practices may fail if tree removals are not timely, elms on private property and wild elms are not included in sanitation programs, and felled elms with bark are saved for firewood. Lanier (1981) and O'Callaghan et al. (1980) devised a trap-tree technique to overcome these problems and to eliminate large numbers of in-flight adults of *S. multistriatus* and *H. rufipes.* Diseased or unwanted elms are treated with the herbicide cacodylic acid or monosodium arsenate by topical application to ax frills, in chain-saw cuts, or by low-pressure injection into buttress roots. *S. multistriatus* and *H. rufipes* are strongly attracted to treated trees but usually the broods they produce largely or entirely fail to mature because of the rapid drying of the bark induced by the treatment. Subject to community approval, trees can be left standing without posing a threat to other elms. The problem of dealing with elms in green spaces, where tree removal is impractical because of the expense involved or the lack of access, might be resolved by this technique (Lanier 1981).

As the result of studies in northern Britain, O'Callaghan and Fairhurst (1983) have concluded that the future for the use of pheromone-baited trap trees remains uncertain. Treated trees did not absorb large numbers of beetles in their studies, but treatment did prevent colonization. Elimination of colonization could reduce disease spread, particularly in areas where other DED control procedures are impossible or impractical.

The Pathogen

Antagonistic agents. A number of agents are known to affect *C. ulmi,* including antibiotics, bacteria, and fungi. Some antibiotics have been observed in the laboratory to be detrimental to the fungus. Clavacin, actinomycin (Waksman and Bugie 1943), candicidin (Lechevalier et al. 1953), and cerulenin (Nickerson et al. 1982) all affect the growth of the fungus. In a study on

binary mixtures of antibiotics, it was observed that each component of the mixture was more efficacious in terms of concentration than when used singly, as long as the following condition was met: one component of the mixture, e.g., tropolone, should insure that the fungal wall was made more permeable, allowing the associative antibiotic, e.g., polyoxin D., to enter and exert its action. This was also true for antibiotics such as puromycin, which singly were not effective against *C. ulmi.* In combination with tropolone, puromycin entered the cell wall and produced a fungicidal effect. The conclusion reached was that a wide number of antibiotics would be effective against the fungus as long as the permeability of the fungus was increased (Mazzone 1985).

The results of antibiotics in retarding DED in the field have not produced optimism. However, this drawback may be attributed to how the antibiotic is administered to the elm. When injected systemically, deposition of the antibiotic throughout the tree may not be guaranteed because of adsorption to tissues, or decay of the antibiotic's potency because of *in vivo* factors. More attention should be given to safeguarding against these defects through the use of such techniques as encapsulation, which is utilized in drug delivery systems (Lim 1984).

In a number of experiments bacteria have been reported as being capable of lysing the cell wall of various fungi (Mitchell and Alexander 1962; Potgieter and Alexander 1966; Skujins et al. 1965; Howard and Gupta 1971; McDonough et al. 1973; Moore et al. 1975). In these studies the mechanisms leading to the disruption of the fungal wall involved chitinase and gluconase systems.

Bacteria may also be *induced* to become chitinolytic. Dubois (1977) grew bacteria in a medium containing chitin as the sole carbon source. The bacterial progeny became chitinolytic, having become induced to elite chitinase and possibly other enzymes. The ability of similarly induced bacteria to disrupt the cell wall of *C. ulmi* has been studied (Mazzone et al. 1982). Strains of *Bacillus thuringiensis*, a dendrolinus variety and a kurstaki variety (Dulmage 1981), were grown in a liquid medium containing chitin as the sole carbon source, and mineral salts (Dubois 1977). The bacterial progeny were chitinolytic, capable of breaking down the cell wall of *C. ulmi,* as evidenced by significant zones of inhibition in culture plates. In theory, the lytic capability of the induced bacteria should last for a relatively long period of time, unless a carbon source more accessible than fungal chitin is available to the bacteria. Field testing remains to be done to test this hypothesis as well as to determine whether the chitinolytic bacteria would be selectively toxic to *C. ulmi,* without affecting the cellulose and pectin constituents of the elm.

The natural antagonism between *C. ulmi* and other fungi has not been adequately exploited. Gibbs and Smith (1978) observed that *Trichothecium roseum* which produces the antibiotic trichothecin (Freeman and Morrison 1949), and *Gliocladium roseum* a noted mycoparasite (Barnett and Lilly 1962) were antagonistic to *C. ulmi.* Gibbs and Smith (1978) noted that *P. oblonga* may be regarded as a principal antagonist of the DED fungus. *Botryosphaeria stevensii* while not colonizing the bark of dying elms to any notable degree, had in inoculation experiments, demonstrated primary antagonism to *C. ulmi.* Gemma et al. (1984) demonstrated that three species of entomogenous fungi, *B. bassiana, M. anisopliae,* and *Nomuraea rileyi* were inhibitory toward both strains of the DED fungus. The fact that fungi antagonistic to *C. ulmi* often do not survive in the confines of the elm may also require encapsulation techniques in order to preserve the spores of the antagonistic fungi.

Search for disease-causing agents in C. ulmi. It has been well established that viruses infect fungi (Hollings and Stone 1971), and a search for viruses in *C. ulmi* was proposed (Brasier and Gibbs 1975). Fungal viruses contain double stranded RNA (Ellis and Kleinschmidt 1967), and Pusey and Wilson (1979) reported the presence of double stranded RNA in all non-aggressive strains of *C. ulmi* tested. This type of RNA was also reported in strains intermediate in aggressiveness, but was found only in a few aggressive strains of *C. ulmi.* Hypovirulence of the non-aggressive strain was presumed by Pusey and Wilson (1979) to be the result of a fungal virus. However, Hollings and coworkers (1974, 1975) were unable to detect virus-like particles

from purified shake cultures of either the aggressive or non-aggressive strain of *C. ulmi* using density gradient and analytic ultracentrifugation, electron microscopy of ultra-thin sections, or serologic tests for double stranded RNA.

Based on light and electron microscopy, Gowen and Manion (1979) described the presence of bacterial-like particles, 0.1 - 0.3 μm, in association with *C. ulmi* in culture. These particles were observed in non-aggressive and weakly to moderate virulent isolates, but not in aggressive isolates of the fungus. Gowen (1983) has recently likened such particles to be related more to spiroplasmas.

Brasier (1983) reported a cytoplasmic disease in the aggressive strain of *C. ulmi* transmitted between mycelia by hyphal fusion. Healthy cultures were transformed by the disease becoming severely reduced in growth and in reproductive fitness. Based on its cultural properties and transmission characteristics, Brasier believes that the transforming factor (d-factor) may be likened to a double-stranded RNA-associated hypovirulence phenomenon. Because of the potential hazard of the aggressive strain of *C. ulmi* overriding populations of the non-aggressive strain, Brasier considers that the attenuation of the aggressive strain may be brought about by the d-factor or a similar infection. The result could be a critical reduction of fitness in the aggressive strain population , similar to that which has occurred with hypovirulence in chestnut blight. The opportunity for spreading the d-factor to healthy isolates of *C. ulmi* residing in elms would take advantage of the vectoring of the fungus by the elm bark beetles. Brasier confirmed that healthy, genetically-marked isolates *C. ulmi* introduced into diseased bark could acquire d-factor infection from other isolates in the bark. Beetles reentering bark of dying elms to breed would again introduce d-infected spores, which would colonize the breeding galleries. Whether this promotion of d-factor in the field would prove promising as a means of controlling DED is under investigation.

Biotechnological Studies

The potential of some new biotechnological procedures can now be applied to the fungus, the vectors, and the elm. Because efforts to find pathogens of the beetles have been essentially futile, it has been suggested that genetic engineering be used on microorganisms found in the microflora of the vectors to change them from harmless to pathogenic microbes (Mazzone 1982). In this connection, Burgess et al. (1979) examined the intestinal microflora of *S. scolytus* at all stages of its development. It was concluded that larval guts of the insect contained essentially one organism, a *Streptomyces* species, possibly, *S. annulatus*. Fungi identified from larvae and pupae included, in addition to *C. ulmi, G. roseum* and *T. roseum*. As noted above, Gibbs and Smith (1978) reported antagonism between these fungi and *C. ulmi, G. roseum, T. roseum,* and the *Streptomyces* species, as well as microorganisms found within the bark of elms could represent potential antagonists of the DED fungi. In industrial firms involved in such experiments, the idea is already being implemented to modify microorganisms in a given environment to make them pesticidal (Baum 1984).

For the pathogen, the genes responsible for pathogenicity could be elucidated. Such experiments could lead to a mechanism whereby the aggressive character of *C. ulmi* strains could be attenuated. Such experiments are underway with other pathological fungi (VanEtten and Barz 1981; Yoder 1983). The attenuation of the genome could lead to a decrease in resistance to antibiotics, which for fungi is an all important consideration, since they, more than any other types of organisms, are responsible for a great number of tree diseases.

While the application of recombinant DNA technology is just beginning for the eukaryotes, including fungi, certain basic procedures are recognized as being essential (Bahl 1981): a) A DNA vehicle (vector, replicon) that can replicate after foreign DNA is inserted into it; b) A DNA molecule to be replicated (passenger); c) A method of introducing a passenger into the vehicle; d) A means for introducing the vehicle carrying the passenger into a host organism in which it can replicate (DNA transformation or transfection); e) A means for screening or genetic selection for those cells that have replicated the desired recombinant molecules. This screening or selection process provides a route to recovery in pure

form of the recombinant DNA of interest. The above methodology has been applied successfully to a number of fungi. Yeasts, for example, have been transformed with yeast DNA sequences of recombinants grown in the bacterium *Escherichia coli* (Hinen et al. 1978). In a similar fashion, such procedures should be tried with *C. ulmi.*

Durzan and Lopushanski (1975) propagated American elms from callus tissue derived from cell suspension cultures. Suspensions established from callus were plated onto agar, where, after transfer to a simpler defined medium over 18 months, shoots were produced. The shoots were removed from the callus, treated briefly with indole-3-butyric acid, and transferred to a sphagnum moss-sand mixture for rooting. Other than the absence of cotyledons, plants from callus were comparable to elm seedlings. Durzan and Lopushanski regarded their experiments as offering a way for the mass propagation and gene conservation of disease resistant species that are desirable for urban forestry.

Photoplasts have been isolated from tissues of a great many plant species (Gamborg and Holl 1977) since the advent of enzymatic digestion of plant cell walls (Cocking 1960). The technoogy has advanced to the point where entire plants can be regenerated from individual protoplasts (Takabe et al. 1971). Of prime importance is the first hybrid plant regeneration by protoplast fusion between sexually incompatible species (Melchers et al. 1978). Redenbaugh et al (1981) are concerned with hybridizing sexually incompatible *Ulmus* species. In this manner they hope to incorporate valuable disease-resistant genes, such as those of *U. pumila* and *U. parvifolia* into the genome of the disease - susceptible *U. americana.* They have shown that it is possible to isolate protoplast from *U. americana, U. pumila* and *U. parvifolia* as well as to demonstrate successful protoplast fusion between *U. pumila* suspension culture protoplasts. In addition, cell wall regeneration and division from some protoplasts occurred.

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ABSTRACT

DAVIS, RICHARD K. 1984. **How a tree is replanted affects its chances for survival.** Am. Nurseryman 160(9): 83-85.

In selecting a tree for transplanting, installers should attempt to match the soil in the root ball to the soil at the site. The "worst match" is a ball with sandy soil and a site that is clay. Planting a ball of clay soil into sandy soil is less of a problem, but there may be difficulties later in getting water into the ball, especially during the first year. In digging the planting hole, installers should ensure that its sides are rough and irregular, which gives the roots the best chance possible to break out of the hole. Smooth, "polished" sides encourage root spiraling. It is important to replant a tree as soon as possible after it is taken from the nursery. When a tree is out of the ground, efforts should be made to protect the root ball. If they are properly used, tree spades will move large trees well.