

LYSIS OF FUNGAL PATHOGENS
BY TREE PRODUCED ENZYMES -- A POSSIBLE
DISEASE RESISTANCE MECHANISM IN TREES

¹
Philip M. Wargo

Plant pathogens produce enzymes that dissolve the host cell wall and cause disease (Albersheim et al. 1969). Just as pathogens can produce enzymes that degrade host cell walls, plants can also produce enzymes that can dissolve the cell walls of pathogens. Enzymes that break down chitin and glucans, major components of fungal cell wall, have been found in bean leaf extracts (Phaseolus vulgaris) (Abeles et al. 1970). Similar enzymes were produced by tomato plants in response to attack by the tomato pathogen, Verticillium albo-atrum (Pegg 1973, Pegg and Vessey 1973). Host plant produced enzymes have also been demonstrated in anthracnose disease of bean caused by Colletotrichum lindemuthianum

But in this case the fungus secreted a protein-containing compound that inhibited host enzymes capable of attacking the pathogen, thereby allowing the fungus to invade host tissues and cause disease (Albersheim and Valent 1974).

¹ Research Plant Pathologist, Forest Insect and Disease Laboratory,
Hamden, CT 06514.

A similar mechanism may exist in trees. Enzymes that break down chitin and glucan have been extracted from root and stem tissue of white oak, Quercus alba; black oak, Q. velutina; red oak, Q. rubra; and sugar maple, Acer saccharum (Wargo 1975). Concentrated preparations of this enzyme mixture caused some dissolution of the cell wall of Armillaria mellea, a root infecting fungus that attacks stress-weakened trees. This enzyme system may be, at least in part, the mechanism of resistance that prevents attack by this fungus in healthy trees. It may also be the system that is altered, inhibited, or destroyed when a tree is stressed, allowing a fungus or fungi to penetrate and/or develop in host tissue.

Further work with the enzymes in sugar maple seem to support this suggestion. Enzyme activities in the sap of defoliated trees was significantly lower than that in undefoliated sugar maple trees indicating that stress does affect the enzymes in the tree (Table 1). Differences occurred in the chitinase activities but they were not statistically significant.

Some defoliated trees still had high enzyme activities and perhaps were able to resist colonization by opportunistic organisms. These trees were the ones that had survived three years of defoliation. Variation among the undefoliated trees showed that enzyme activity in several of these trees were as low as or lower than the stressed trees. These low level trees may represent the trees that would be influenced by stress and become susceptible to attack by secondary organisms such as A. mellea.

Fungi that infect the twigs and branches of trees could also be affected by such an enzyme system. In our work with defoliation and sugar maple, Dr. David R. Houston and I (1974) observed significant twig and main stem colonization of defoliated trees by Stegonosporium ovatum. The fungus was often found girdling small branches and the upper portions of the main stem on young maple trees causing the foliage to wilt and die. This fungus can also be lysed by chitin and glucan degrading enzymes (Table 2), other stem infecting fungi of oak (Schmidt and Fergus 1965), birch (Hahn and Eno 1956), and ash (Silverborg and Brandt 1957) may be affected in a like manner. These fungi usually do not attack healthy or unwounded tissues and predisposition of the tissues by some stress is necessary for infection.

In addition to fungi, insect attack such as borer activity could be affected by these enzyme systems. The exoskeleton of all stages of these insects (larvae and adults) contains chitin. Extra-insect sources of chitin could influence exoskeleton formation.

Dieback and decline diseases of trees are some of the most serious problems with northeastern trees. Thousands of trees die annually in our forests, woodlots, backyards, and front yards. And it is difficult to prevent the initiating factor - stress. One obvious fact about these stress-related-diseases is that not all trees succumb even though they are subjected to the same degree of stress and are in the presence of the opportunistic secondary organisms. These trees are tolerant of the stress and/or resistant to attack by these organisms. This mechanism

Table 1.--Effects of defoliation (3 consecutive years) on activity of β -1 3-glucanase in the spring, sap of sugar maples.

Defoliation treatment	Number of trees	Average glucanase activity	Range of activity
No defoliation	10	.16 ^{a*}	.07 - .22
May defoliation	7	.09	.04 - .12
June defoliation		(All trees had died)	
July defoliation	8	.07	.02 - .11
August defoliation	10	.09	.04 - .17
s ^b = .039 * -P = .01			

^a μ moles of glucose equivalent produced in one hour at 38°C: Reaction mixtures contained .5 ml sugar maple sap concentrated 10 fold with an ultra filter (*AMICON PM30), 1.5 ml 5.0% Na acetate buffer, and .5 ml of 1 percent laminarin. (See Wargo, P. M. 1975).

^b Standard deviation with 30 d.f.

* AMICON - Mention of a particular produce should not be taken as an endorsement by the Forest Service or the U. S. Department of Agriculture.

Table 2,--Dry weight loss of prepared hyphal wall of *Stegonosporium ovatum* challenged with glucanase and chitinase for 120 hours at 38 degrees C

Treatment	Weight loss	Reducing sugar	N-acetylglucosamine
Endo-glucanase	17	31 ^b	--
Exo-glucanase	22	51	--
Chitinase	2	--	26 ^b
Endo + Exo	22	63	--
Endo + Chitinase	22	22	3
Exo + Chitinase	20	71	4
Endo + Exo + Chitinase	22	70	5

^a Enzymes obtained from cultures of *Rhizopus arrhizus* (QM1032), an unidentified basidiomycete (QM806), and a bacterium, *Serratia marcescens*, (obtained from E. T. Reese, Pioneering Research Lab, U.S. Army, Natick Laboratories, Natick, Mass.) were prepared according to Reese and Mandels (1959) and Monreal and Reese (1969).

^b Expressed as percent of the material dissolved.

of resistance should be identified and exploited if possible to improve our trees and make them less susceptible to these dieback and decline agents.

Host produced enzymes capable of attacking pathogenic organisms is a possible mechanism for this observed resistance; and efforts to determine if and how this system works should be continued.

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