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George R. Stephens measures tree in State Forest

Pesticides protect hemlocks from adelgid

Spring weather favors dogwood anthracnose

Station purchased first State Forest land

New form of enzyme discovered

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Pesticides will protect ornamentals from hemlock woolly adelgid

By Mark S. McClure

During the past 6 years a tiny aphid-like insect has become a serious pest of forest and ornamental hemlock trees in Connecticut. The insect is the hemlock woolly adelgid, *Adelges tsugae* Annand, so named because for most of its life it is covered with a secreted woolly substance (Fig. 2). This adelgid occurs primarily on the young branches where needles are attached. It sucks sap and probably also injects a toxic saliva during feeding. This causes rapid desiccation and discoloration of foliage and death of the tree usually within 4 years. Both forest and ornamental trees have been killed.

Hemlock woolly adelgid occurs both in eastern and western North America. It was first observed in the West in Vancouver, BC in 1922 and now occurs from northern California to southeastern Alaska. This same adelgid was first reported in the East in Virginia in 1951 and now occurs from North Carolina to southern New England including most of Connecticut and Rhode Island and parts of southern and eastern Massachusetts. My studies have shown it to be cold-hardy and to be dispersed by wind, birds, and forest-dwelling mammals and by humans during nursery, logging and recreational activities. Therefore I expect the adelgid will probably continue to spread northward into the more contiguous hemlock growing areas of northern New England and southeastern Canada.

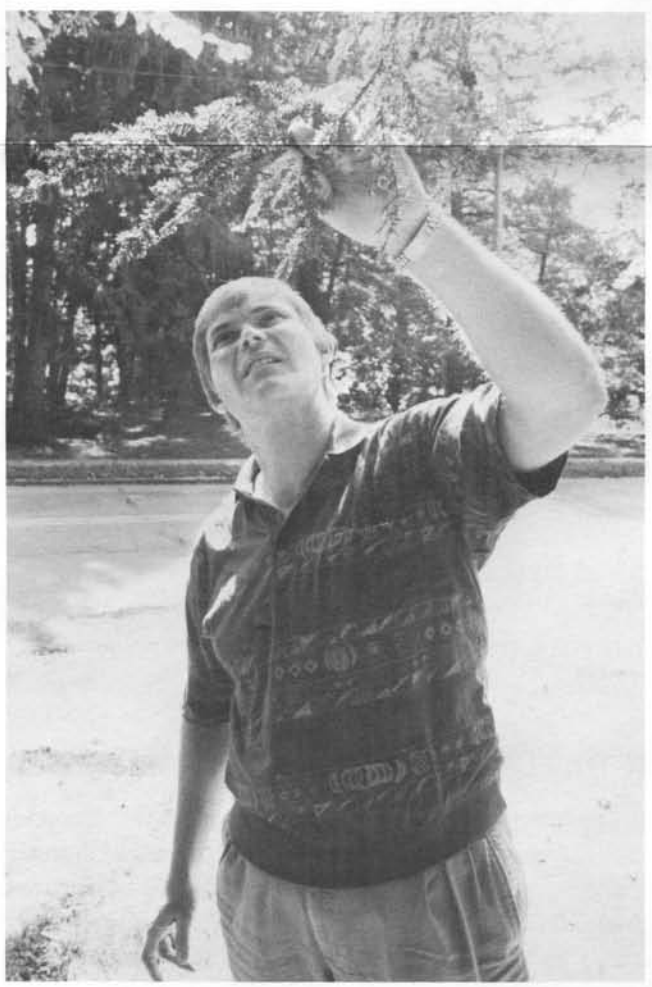


Figure 1. Mark S. McClure examining an infested hemlock.

Table 1. Effectiveness of chemical pesticide sprays for controlling hemlock woolly adelgid.

Pesticide (ai;oz/100 gal)	Application period	Adelgid mortality %
Horticultural Oil		
(2%)	March-April	95
(1%)	May-September	100
(2%)	October-November	100
Insecticidal Soap (250)	May-November	100
Diazinon (25)	May-November	99
Fluvalinate (1.25)	May-November	100
Malathion (16)	May-November	99
Oil (1%) + Ethion (0.9)	May-November	100
Unsprayed		8

Hemlock woolly adelgid was probably introduced accidentally into North America from Japan. In Japan it is a harmless inhabitant of several hemlock species. In the northeastern United States, however, it usually attains high damaging numbers and has killed thousands of eastern and Carolina hemlocks during the past 40 years. This contrasts with the situation in western North America where no significant damage to forests of western and mountain hemlock has been noted.

Because no natural controls have yet been found, I have investigated chemical control of the adelgid. In nursery and landscape plantings a number of pesticides have been successful. A backpack or garden hose sprayer may be sufficient to drench smaller trees but those taller than 20 to 30 feet may require a high-pressure hydraulic sprayer. It has not been necessary to target particular life stages of the adelgid; all stages are susceptible, and pesticide applications can be made throughout the year as weather permits (Table 1).

I found that thorough coverage with these pesticides killed 95 to 100% of the adelgids compared to only 8% dead from other causes on unsprayed trees. Horticultural oil and insecticidal soap have become the pesticides of choice among arborists, groundskeepers and the general public because they are highly effective, relatively specific to the target pest, and are relatively safe to the environment.

In other studies I found that microinjection and implantation of concentrated chemical pesticides into the stem of infested hemlocks provides excellent control on very large trees inaccessible to spray equipment or where spraying is undesirable such as near waterways and recreation areas (Table 2). The Mauget Microinject System (J.J. Mauget Co., Los Angeles, CA) involves drilling small, shallow holes into

Table 2. Effectiveness of injected and implanted pesticides for controlling hemlock woolly adelgid.

Treatment	% Dead adelgids After 4 weeks	After 20 weeks
Microinject		
Bidrin	94	70
Metasystox-R	98	88
Implant		
Acephate	93	60
Untreated	27	49

the root flares at the base of the tree and inserting pressurized plastic capsules containing concentrated liquid pesticide. The pesticide moves into and up the tree where it is intercepted by the feeding adelgids. The CSI Implant System (Creative Sales Inc., Fremont, NE) involves drilling larger, deeper holes in a spiral around the trunk of the tree and inserting a plastic cartridge containing a dry powdered pesticide within a gelatine capsule. The sap flow of the tree dissolves the gelatine capsule and the pesticide and carries it up the tree where it too is ingested by feeding adelgids.

I found that injections of bidrin and metasystox in May killed more than 94% of the adelgids present at the time and more than 70% of the adelgids present 5 months later (Table 2). Similarly implants of acephate in May killed 93% of the adelgids present then and 60% of those present after 20 weeks. Natural mortality of adelgids was less than 50% during this period.

Feeding trees with nitrogen is common among nursery growers, arborists and landscapers to improve the growth and appearance of hemlock and presumably to increase their resistance to pests. Several years ago I discovered to the contrary that nitrogen fertilization of hemlock actually enhanced the survival and fecundity of a similar adelgid-like insect to the detriment of the tree. Therefore I recently evaluated the effects of fertilization on the adelgid and the growth and appearance of hemlock.

Figure 2. Egg masses of hemlock woolly adelgid (*Adelges tsugae*) on eastern hemlock.

I evaluated nitrogen by soil application, by Mauget microinjection, and by CSI implantation either alone or in combination with pesticides. When used alone, fertilization neither improved the appearance and growth of infested hemlocks nor made uninfested ones more resistant to adelgid attack. When applied in combination with pesticides, fertilizer reduced their effectiveness by enhancing adelgid survival and reproduction. Percent survival of adelgids and numbers of eggs produced per adult were twice as high or more on fertilized hemlocks than on unfertilized ones. Therefore fertilization enhances growth only of uninfested hemlocks.

I have been trying to determine if the two western and one Asian hemlock species are more resistant to the adelgid than our two native hemlock species in the East. Hemlocks obtained from nurseries in Connecticut, North Carolina and Oregon were planted in 1989 at The Experiment Station's Lockwood Farm in Hamden. These were artificially infested with egg masses of the adelgid in 1990, and I have recorded adelgid survival and fecundity (number of eggs produced) and the number of hemlock buds which had been killed.

Significantly fewer adelgids survived and adults produced significantly fewer offspring on their native Asian hemlock and on the two western North American species than on the two eastern North American species. In addition there was little or no damage to the Asian and western hemlocks while damage to the two eastern species exceeded 85% of the buds killed (Table 3). Therefore, western hemlock may be a good replacement species. We are now investigating how well western hemlocks will grow in the East over the long term.

Most damage to hemlock has occurred in forests and mature landscape plantings where trees are large, tightly packed and inaccessible to spray equipment. In these areas thorough coverage of trees with pesticide sprays is not possible and native natural enemies have not been effective biological control agents.

Hope for biological control of this introduced pest probably rests upon the discovery of an effective natural enemy in Asia and its successful introduction and establishment in North America. Until then the hemlock woolly adelgid can be controlled easily in ornamental and landscape settings using sprayed, injected or implanted chemical pesticides.

Table 3. The performance and damage of hemlock woolly adelgid on hemlock (*Tsuga*) species growing in Connecticut.

Hemlock species	Adelgid survival %	Number eggs per adult	Hemlock buds killed %
Asian			
T. diversifolia	1	44	0
Western			
T. heterophylla	4	56	1
T. mertensiana	2	52	0
Eastern			
T. canadensis	83	108	94
T. caroliniana	78	94	86

Spring rainfall and cool temperatures favor development of dogwood anthracnose

By Victoria L. Smith

Native flowering dogwood (*Cornus florida*) is a popular landscape tree throughout the eastern United States. Its early spring display of pink or white flower-like bracts is an eagerly-awaited event, and many communities, both large and small, host Dogwood Festivals to celebrate this harbinger of spring. It is also a valuable woodland understory tree, providing cover and edible seeds for a number of animals and birds. However, there is a fear that the dogwood will go the way of the elm and the chestnut, and disappear from our landscape, due to a fungal disease called dogwood anthracnose.

Dogwood anthracnose was first described in the late 1970's, both in New England and in the Pacific Northwest. The fungus may be an introduced pathogen, because its presence was not recorded prior to that time. The fungus has not been adequately described to give it an accepted scientific name. The disease is now present throughout most of the natural range of the flowering dogwood. It also can occur on another popular landscape dogwood, the kousa dogwood, *Cornus kousa*, but the effects are not as devastating.

The most obvious symptom of dogwood anthracnose is a leaf blight, seen as purple-bordered lesions with tan centers on the leaf (Fig. 2). Within the tan centers, fruiting bodies of the fungus form; they look like tiny orange to black specks. Spores of the anthracnose fungus can be spread from the fruiting bodies by splashing water. In cool wet weather, the purple and tan lesions increase in number and they expand rapidly, causing leaves to shrivel and die.

Figure 1. Victoria L. Smith and a dogwood tree.

Leaves killed by anthracnose infection frequently hang on the twig for one season or more, and may serve as a source of fungus spores for newly-emerging leaves. Leaf infections can also progress down through the petiole and into the twigs, causing a twig blight. Infected twigs turn purple and die, with more fruiting bodies of the fungus forming on blighted twigs. The fungus has been reported to produce toxins, which are involved in the formation of cankers on the main stem of the tree at the base of blighted twigs. The tree is killed when one or more cankers girdle the main stem.

In the southern United States, dogwood anthracnose has been reported to cause lesions on the seeds of *C. florida*. Seed infection may reduce germination of the seeds and viability of the seedlings. Although I have examined hundreds of seeds from many severely infected dogwood trees in Connecticut, I have not yet found a single seed infection.

In addition to causing leaf blight, the anthracnose fungus also can infect the flower bracts, causing unsightly pink to purple splotches. Bract infection can occur on both *C. florida* and on *C. kousa,* which is somewhat susceptible to bract blight. However, since bracts drop off trees rapidly following infection, bract blight is not as serious as leaf blight.

The leaves of kousa dogwood are highly resistant to anthracnose, and hybrids of *C. florida* and *C. kousa* have recently become available at selected wholesale nurseries. Plant breeders anticipate that these hybrids are resistant to anthracnose, but the trees have yet to be tested in a controlled experiment. The hybrids have characteristics of both parent trees; that is, some have floral characteristics similar to *C. florida*, while others have a shape and branch structure similar to *C. kousa*. Perhaps some hybrid trees will be suitable replacements for dogwoods that have succumbed to anthracnose.

The effects of infection by the anthracnose fungus can be reduced by using a few simple cultural measures to increase tree vigor. Dogwoods are shallow rooted, and greatly benefit from 1-2 inches of water per week, especially during the hot summer months. Care must be taken, however, not to wet the leaves, as this possibly could spread spores of the anthracnose fungus to uninfected leaves. Mulch, such as wood chips, will aid in conserving soil water, and will protect the base of the tree from cuts inflicted by lawn mowers or string trimmers. Pruning of infected twigs and removal of infected leaves will improve the appearance of the tree and will remove some of the anthracnose fungus from its vicinity. Application of fertilizer in the early spring will also boost tree vigor. In addition, two fungicides, benomyl and chlorothalonil, are currently registered for use on dogwoods for anthracnose. Application of fungicides must begin as soon as possible after leaf emergence in the spring and be done at regular intervals thereafter.

Figure 2. Anthracnose on a dogwood leaf.

These cultural measures will enhance the tree's ability to withstand the disease, and they may remove the need to spray.

In past years, it was difficult to find a tree in Connecticut that was not infected with anthracnose. Many were severely defoliated, and cankers were common on the branches and trunk. In some areas, death of trees within one summer was not unusual. Previous investigators assumed that summer droughts and colder than usual winters were weakening the trees and exacerbating the effects of the disease, but the exact cause of tree mortality was not clearly understood.

I have been collecting data on severity of naturally-occurring epidemics of dogwood anthracnose in Connecticut in the past 2 years. My experimental plots are located in: New Haven (Edgehill Rd.), Hamden (at the Experiment Station's Lockwood Farm), Westchester (Salmon River State Forest), and North Branford (Lake Gaillard). At weekly intervals, I collected fifty leaves from trees at each of these four sites, and each lesion on each leaf was counted and its length and width measured. Number of lesions per leaf was used as a measure of disease incidence. Total surface area of each leaf was then measured with a leaf area meter. The percentage of the leaf area diseased was then calculated and used as a measure of disease severity.

Last year at the Hamden site, disease severity peaked early in the season at almost 4.5% of the leaf area on July 3, 1990. After that time, daily maximum temperatures were frequently above 90F, and diseased leaf area was consistently lower than on July 3. Rainfall was near normal during May and June 1990. Perhaps the high daily temperature suppressed lesion development after the first week of July.

In the 4 weeks following leaf emergence, the number of lesions on the leaves, disease incidence, increased. However, in the following weeks, the number of lesions on the leaves did not increase significantly, indicating that most infections may occur only early in the season. Therefore, it may be necessary to apply chemical control measures only during this narrow "window" of susceptibility.

The number of lesions on the trees did not increase much after the first 4 weeks following leaf emergence in 1991. At the Hamden site, disease severity has consistently been less severe than in 1990; the maximum through September has been less than 0.8% of the leaf area. Perhaps the early season drought and high temperatures during the summer have restricted lesion development. Disease severity and incidence at the other three sites were similarly less this year. These results indicate that the dogwood anthracnose fungus has only one chance to cause infection in each year, and that is soon after leaf emergence.

I remain optimistic about the future of the dogwood in our landscape and forests.

Experiment Station purchased first state forest and park land

By George R. Stephens

You may ask, "How in the world did the Station ever become involved in acquisition of forest and park land?" It all came about in June 1901 when the General Assembly passed "An Act Concerning the Reforestization of Barren Lands". This act required the Station's Board of Control to appoint a State Forester to serve at the pleasure of the Board, to have an office at the Station in New Haven, and to receive no compensation other than his regular salary as a member of the Station staff. Walter Mulford became the first Station Forester in April 1901, and in July he was appointed the first State Forester.

The act further stated, "The state forester is authorized to buy land in the state suitable for the growth of oak, pine, or chestnut lumber at a price not exceeding four dollars per acre to the amount of the appropriation hereinafter named, which land shall be deeded to the State of Connecticut and shall be called a state park." The sum of $2000.00 was appropriated for the two fiscal years ending September 30, 1903.

Mulford clarified the term "park" at the annual meeting of the State Board of Agriculture in December 1901. He said, "Calling it 'State Park' is perhaps misleading to many people. The idea at present is not that it shall be primarily a game or fish preserve, or a place of great natural beauty, but that it shall be used as a demonstration area for giving object lessons in the practical working of the principles of forestry."

To alert landowners to the Station's interest in purchasing land in 1901, Mulford distributed throughout the state a circular entitled, "Notice to Owners of Waste Lands and Cutover Woodlands Suitable for Growth of Timber." In it he cited the act concerning reforestization of barren lands, the intention of the state to pay taxes on the land, and the goal to manage the land so as to secure as rapid and profitable growth of timber as possible. Mulford wrote, "It is hoped that this undertaking may be practically useful in restoring to forest production some land at present nearly worthless, and that such land may be so tended as to serve as an object lesson in tree planting and in the proper management of woodland, thus leading to a more rational and consequently more profitable handling, by their owners, of the cord-wood lands and timber lands of Connecticut." Mulford advised that offers of land would be accepted through November 10, 1901.

Twenty-three landowners offered to sell to the Station 5500 acres scattered in 21 towns, at prices of $1.00 to $4.00 per acre. Mulford examined these parcels during the winter and spring of 1901-02. Subsequently, Mulford examined an

additional 2500 acres and indicated five possible sites for the state park. The first state park of 698.5 acres in Portland was purchased in 1903 for $1110.12 at prices ranging from $1.00 to $2.38 per acre. Because planting costs were limited to $2.50 per acre, Mulford was unable to plant on these lands.

The 1903 Forestry Act appropriated $2000.00 for the work of the State Forester and authorized him to make thinnings in the land purchased. Returns from sale of products were to be devoted to the maintenance and care of the forest, the restriction of $2.50 per acre for planting was removed, and the term "state park" was changed to "state forest".

In 1904 Austin F. Hawes succeeded Mulford as Station and State Forester. In 1905 he purchased an additional 288 acres in Union and in 1908, 130 acres in Simsbury. By 1909 additional purchases increased the Portland State Forest to 1100 acres. The Annual Report of the Station for 1906 stated, "State forests have thus been started in Middlesex and Tolland counties. It is the Station's policy as soon as funds become available to establish similar tracts in the other counties of the state, so that all land owners will have easy access to these examples of forestry."

Prior to 1909 the General Assembly had appropriated $8000.00 for the purchase, protection, planting, and payment of taxes to towns for land acquired. However, as the amount of land increased, the cost of protection and taxes increased, leaving less funds for subsequent purchases. Therefore, in 1909 the General Assembly appropriated $5000.00 specifically for the purchase of land suitable for state forests and in 1911 it increased the maximum purchase price to $8.00 per acre. However, the salary of the Forester and the cost of correspondence, travel and other expenses incidental to the work of the State Forester continued to be borne by the Station.

In 1925, Station Director E.H. Jenkins wrote, "The policy of buying woodland by the state was frowned upon by the Appropriations Committee in 1909, as I remember it. The chairman was a brick manufacturer with extensive knowledge of woodland who held that the whole business of forestry in this state was impracticable and the holding of such real estate unwise for the state. But he consented to make a trip to Portland with the committee one day in spring to see what was already bought. We did all we could to make the visit agreeable. We had a decent out-of-door lunch, I think cider flowed, and then we walked through the land the state owned. We came across a trout brook. We saw a place where deer had parked in the winter, and been fed at times by the good caretaker Del Reeves, who told of the pheasants and quail which sometimes came to his hen-yard to feed in rough weather. We showed some excellent timber growth along with the stump land and explained that this land, in the center of the state we had got for little over $1.00 per acre. The committee's hearts were touched by the trout brook and the protected game birds and other animal life, and their pockets were touched by the timely purchase of cheap wood-producing land. The chairman asked of the forester, aside, if more such land could be bought and his attitude toward state forests was reversed."

In 1909 Samuel N. Spring succeeded Hawes, and during his 3-year tenure he purchased 600 acres in Cornwall. In 1912 Walter O. Filley became Station and State Forester. The Experiment Station Report of 1914 indicated addition of 80 acres to the Portland State Forest and 300 acres to the Cornwall State Forest. The period 1918-21 was an especially active time

Figure 1. A 4-year-old white pine seedling planted in Portland State Forest is taller than caretaker Del Reeves. (1911 photo)

of land acquisition. Annual reports of the Station showed additional purchases of 626 acres in 1918; 254 in 1919; 408 acres in Eastford in 1920; and 174 acres in 1921, 154 in Eastford and 20 in Portland.

Filley was also involved in acquisition of land for parks. In 1913 the State Park Commission was formed and the Station Forester was an *ex officio* member. Since most of the parks were wooded, Filley was called upon to inspect them and to supervise any forestry operations conducted in the state parks. The Station Report of 1919 indicated that the Commission had acquired 4000 acres in its 6 years of existence.

In 1921 an act of the General Assembly created the State Park and Forest Commission and relieved the Station Forester of his duties as State Forester and State Forest Fire Warden. Management of the five state forests was transferred from the Station to the Commission. By that time the Station had purchased 4177 acres at a total cost of $21,441.92. Austin F. Hawes, Station and State Forester during 1904-09, was appointed the new State Forester by the Commission.

Creation of the new commission did not end the Station's role in land acquisition and forest policy, because the same act that created the State Park and Forest Commission also made the Station Forester an *ex officio* member of the Commission, a requirement that continued until 1947.

The names Portland, Union, Simsbury, Eastford, and Cornwall State Forests are no longer used. Portland became the Meshomasic State Forest and Union, the Nipmuck. Eastford State Forest was renamed Natchaug, and Cornwall became the Housatonic. At least a portion of the Simsbury State Forest is now known as Stratton Brook State Park and the remainder,

Massacoe State Forest. Currently, the Department of Environmental Protection manages 30 state forests with 139,377 acres and 58 state parks, mostly wooded, with 30,674 acres.

In 1901 in the First Annual Report of the Forester, Walter Mulford wrote, "The amount of the appropriation is so small and the restrictions imposed so limit its use, that it seems impossible to make with it a suitable beginning of forest work for the people of the State. Yet it is hoped that a little of real value to the State may be accomplished—a beginning which, if it is ever to attain real success, must be put on a far more liberal basis." Fortunately, the restrictions were gradually removed, the basis became more liberal, those responsible persevered, and from the small beginning of 698 acres a great legacy to the citizens of Connecticut grew. The Station is justifiably proud of its role in this early effort to establish the State's forest and park system.

Discovery of new form of enzyme may provide opportunities for plant improvement

By Evelyn A. Havir

Until relatively recently, all catalases were assumed to be similar in properties, but research at the Experiment Station is uncovering new information which indicates that this assumption is incorrect and that there are at least two types of catalase in higher plants. In addition to providing new insights into plant metabolism, recent work suggests changing catalase activity may produce plants with improved characteristics.

Catalase was one of the earliest enzymes identified and studied. In 1901 Oscar Loew wrote in a U.S. Department of Agriculture report, "Since it is clear that the power of catalyzing hydrogen peroxid(e) [decomposition] is not due to any of the known enzym(e)s, it appears justifiable to ascribe this power to a special enzym(e). The writer proposes to call this catalase." Since these initial investigations with catalase from tobacco extracts the enzyme has been extensively studied.

Catalase functions in several important ways in plants. First, it serves a protective function by converting toxic hydrogen peroxide (H_2O_2), produced by the process of photorespiration, to water and oxygen by two reactions. In reaction (1), two molecules of H_2O_2 yield 2 H_2O and O_2 and in reaction (2), one molecule of H_2O_2 is used to oxidize an organic compound such as ethanol to acetaldehyde and water. Second, catalase acts to prevent CO_2 loss from the reaction of H_2O_2 with certain leaf compounds. Work by Kenneth Hanson and Richard Peterson suggests that significant CO_2 loss can occur by this reaction. The presence of high catalase would prevent H_2O_2 accumulation and loss of CO_2, thereby increasing net yield of photosynthesis. This hypothesis is supported by the work of Israel Zelitch who isolated a mutant with oxygen-resistant photosynthesis and which also exhibits higher catalase activity than the wild-type plants.

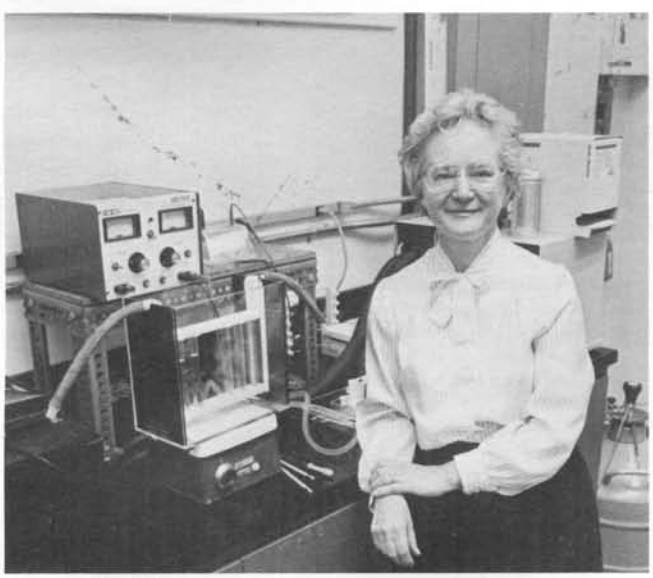
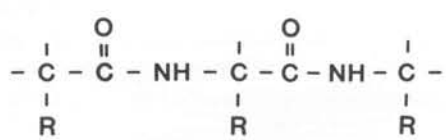


Figure 1. Evelyn Havir with electrophoresis equipment used to detect catalase.

I began studying catalase in collaboration with Neil McHale who was interested in obtaining a mutant of tobacco that lacked catalase. However, if several forms were present, controlled by separate genes, it would be very difficult to obtain a mutant without catalase. I quickly learned by using a technique called chromatofocusing that there were a number of forms or isozymes of catalase in tobacco leaves. Our original method required large amounts of leaves and only two samples could be examined in a day, but recently we have begun to use an electrophoretic technique called isoelectric focusing which allows us to analyze the forms of catalase in 8-10 small samples per day.

Electrophoresis is defined as the migration of charged molecules in an electric field. Enzymes are large proteins composed of many amino acids which have positive and negative groups, allowing separation by electrophoresis according to charge and size. The basic structure of a protein can be written:



where R is a charged group such as COO^- or NH_3^+. In isoelectric focusing, a hydrogen ion (H^+) gradient is generated by high voltage in a solid support called a gel and the proteins or enzymes migrate to the H^+ concentration at which their net charge (sum of positive and minus groups) is zero. After electrophoresis, the gel is treated with specific reagents to detect the presence of enzymes and/or proteins.

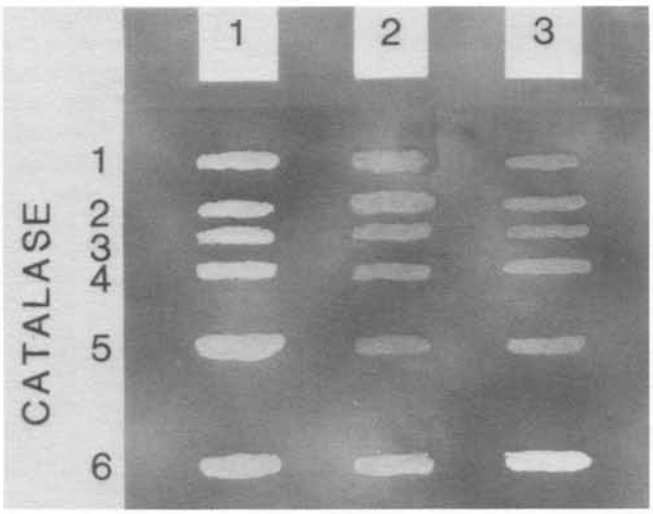


Figure 2. Isoelectric focusing of catalase isolated from tobacco seedlings. (1) Untreated plants grown in air; (2) Plants treated with 3-amino-1,2,4-triazole, grown in air; (3) Untreated plants grown in 1% CO_2. Gel is stained to detect enzyme activity (catalase appears as clear band).

A diagram of an isoelectric focusing gel stained for catalase is shown in Fig. 2. The white areas represent enzyme activity. Thus, in lane 1, at least six bands, representing six forms of catalase, are present in a sample prepared from leaves of tobacco seedlings. If the seedlings are treated with the specific catalase inhibitor and herbicide 3-amino-1,2,4-trizole (AT) before extracting the enzymes, the results shown in lane 2 are obtained. The first five bands have all but disappeared due to inactivation by AT but the sixth is unaffected. In lane 3, the seedlings have been grown in 1% CO_2 instead of air (0.03% CO_2). In this case, the first five bands are reduced and the sixth is intensified. Thus the first five isozymes of catalase are similar to each other in their sensitivity to AT and their response to 1% CO_2 but the sixth is clearly different.

I have also demonstrated a number of other differences between catalases 1-5 and catalase-6. The most important is that catalase-6 can carry out reaction (2), a peroxidatic reaction in which H_2O_2 is used to oxidize a compound such as an alcohol, much more efficiently than catalases 1-5. Catalase-6 is called EP-CAT (enhanced-peroxidatic catalase) and I have also found EP-CAT in such diverse species as barley, spinach and corn. Hitherto the presence in plants of catalase with these properties was unknown.

The next step was to study the properties of EP-CAT. For this, I developed methods to separate or isolate the protein. After each procedure, electrophoresis was used to monitor the results. Large proteins are frequently composed of smaller units called subunits which are loosely bound together. Treatment with detergents disrupts these bonds and coats the subunits with a negatively charged film. Now, during electrophoresis, protein migration is solely a function of size since all are the same charge. In Fig. 3 the use of this technique in characterizing two of our forms of catalase is shown. The separation of a typical complex mixture of proteins is shown in lane 2. In lanes 3 and 4, it can be seen that our goal has been achieved and we have obtained pure samples of catalase-1 and EP-CAT. Furthermore, by comparison with proteins of known size or molecular weight, shown in lane 1, the size of our isolated enzymes can be determined. In this case the subunit of catalase-1 is 55,000 and that of EP-CAT, 53,000. Since we know that each is composed of 4 subunits, it follows that catalase-1 has a molecular weight of 220,000 and EP-CAT 212,000.

Once pure samples of catalase were obtained, antibodies to catalase-1 and EP-CAT were prepared in mice. The antibody to catalase-1 reacted against catalases 1-5 but not against EP-CAT. Also, the antibody to EP-CAT reacted only against EP-CAT and not against catalases 1-5. Thus all our results show that catalases 1-5 are similar to each other and that EP-CAT is unique.

With the discovery of different types of catalase and knowledge of their properties, it is possible to look forward to some applications of our findings. For example, it may be possible to increase the amount of EP-CAT in tobacco by using molecular biology techniques. As described earlier, EP-CAT is more resistant to the herbicide AT than catalases 1-5 and plants containing increased amounts of this form of catalase would probably survive treatment with AT that would destroy weeds. Other applications will arise as we learn more about the role and function of EP-CAT in plants.

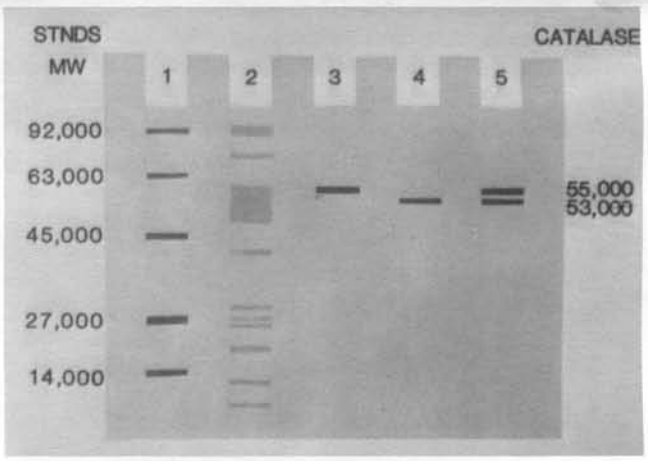


Figure 3. Electrophoresis in the presence of detergent (SDS-PAGE). Gel stained for protein (dark bands). (1) Standards, proteins of known molecular weight. (2) Complex mixture of proteins extracted from leaves. (3) Purified catalase-1. (4) Purified EP-CAT. (5) Mixture of catalase-1 and EP-CAT.

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