FRONTIERS of Plant Science

FALL 1997
Volume 50 No. 1

James A. LaMondia (L) and Donald E. Aylor (R) inspect a leaf covered with blue mold lesions

Treatment purifies water of wood preservatives

Blue mold disease returns to the Connecticut Valley

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION, founded in 1875, is the first experiment station in America. It is chartered by the General Assembly as an independent State agency governed by a Board of Control. Station scientists make inquiries and experiments regarding plants and their pests, insects, soil and water quality, food safety, and perform analyses for State agencies. Factual information relating to the environment and agriculture is provided freely and objectively to all. The laboratories of the Station are in New Haven and Windsor; its Lockwood Farm is in Hamden. Copies of this and other publications are available upon request to Publications; Box 1106; New Haven, Connecticut 06504

http://www.state.ct.us/caes

ISSN 0016-2167

New treatment purifies water of wood preservative contaminants

By Joseph J. Pignatello and Margaret Engwall

Several years ago, we developed a process (U.S. Patent No. 5,232,484) for destroying pesticides in water. This process uses hydrogen peroxide combined with an iron catalyst and solar ultraviolet light to generate a reactive chemical entity known as the hydroxyl radical. This radical rapidly oxidizes pesticide molecules, efficiently breaking them down to carbon dioxide, water, and harmless inorganic salts. The photo-assisted Fenton, or photo-Fenton, process is a series of reactions that produce the hydroxyl radical. We have now extended its scope to the degradation of chemicals commonly found in groundwater at coal-tar and creosote contaminated sites. We have shown that such chemicals are destroyed and the toxicity of the treated water is eliminated.

Coal tar is a viscous liquid formed as a byproduct of coal gasification. Coal gas was used mainly as a fuel for heating and lighting in many industrialized countries until the early 1900's when it was replaced by natural gas. Since there was no use for coal tar at the time, it was disposed on-site in waste pits. Many of these waste pits were not contained, thus allowing groundwater and surface water to come in contact with the coal tar. There are thousands of these sites in the United States.

Creosote is an oily material, similar in composition to coal tar, that is manufactured from coal for use as a wood preservative in, for example, telephone poles, fence posts, railroad ties and pier pilings. Creosote is often combined with another wood preservative, pentachlorophenol. As of May 1997, there were 59 wood treatment facilities using creosote and/or pentachlorophenol listed on the National Priority List compiled by the U.S. Environmental Protection Agency (EPA) to identify hazardous waste sites of high priority for cleanup. None of these sites is in Connecticut, and only one is in New England. However, the Connecticut Department of Environmental Protection (DEP) is overseeing the cleanup of two facilities in the Greater New Haven area that either produced or stored creosote. Over 700 current or former wood preservative operations have been identified in the United States.

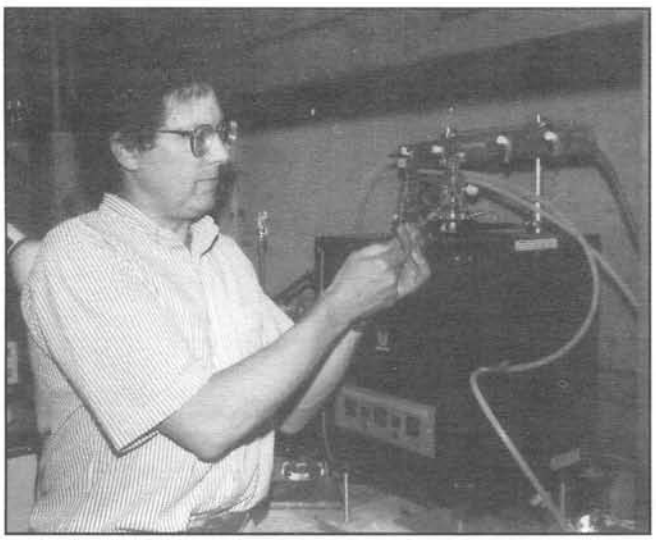

Figure 1. Joseph Pignatello taking a sample for analysis from the photoreactor.

Creosote and coal tar are complex mixtures of organic compounds, many of which are at least slightly soluble in water. Table 1 lists the compounds we identified in a batch of water that was in contact for 7 days with a creosote reference standard. These compounds include polycyclic aromatic hydrocarbons of up to five rings in size; heterocyclic aromatic compounds containing nitrogen, oxygen, or sulfur atoms in the ring; and various phenolic compounds. Figure 2 shows an example of each type of compound. A number of unidentified compounds were also present in the water.

Many of the compounds in Table 1 are classified by the EPA as questionable, suspected or confirmed carcinogens. In addition, minimum acceptable concentrations for groundwater and surface water have been established for some of them by EPA and the DEP, as indicated in Table 1.

Degradation experiments on water samples saturated with creosote and pentachlorophenol were performed in a special glass vessel inside a photoreactor. The photoreactor consists of a rectangular metal box containing a bank of 16 "black light" lamps surrounding the vessel, which is transparent to the light. The lamps emit light at wavelengths in the near ultraviolet region (300-400 nanometer), and the ultraviolet light intensity is comparable to that in midday summer sunlight. Beneath the vessel is a stir plate that agitates the solution during the reaction. The vessel was equipped with ports for removing samples and for purging the mixture with a stream of gas to remove volatile products.

The reaction solutions were prepared by adding to the creosote-pentachlorophenol solution hydrogen peroxide and a ferric salt, adjusting the pH to 2.8, and turning on the lights. Loss of the original contaminants was determined by removing a small sample of the reaction solution at various times, extracting the sample with organic solvent, and analyzing the extracts by gas chromatography. The results

Table 1. Data for pentachlorophenol and the identified components of American Creosote P2 reference standard from the American Wood Preservative Association: their carcinogen classification, whether DEP Water Protection Criteria have been established, and their percent degradation in the photo-Fenton reaction.

Compound	Toxicity Classification	DEP GW Prot. Criteria[a]?	DEP SW Prot. Criteria[b]?	Percent Degradation in 5 min	Percent Degradation in 3 hrs
O-Heterocyclics					
Benzofuran				100%	100%
Dibenzofuran				98%	98%
S-Heterocyclics					
Benzo[b]thiophene				100%	100%
Dibenzothiophene				75%	100%
N-Heterocyclics					
Quinoline				100%	100%
Isoquinoline				100%	100%
Methylquinone (isomer)				97%	96%
Acridine				92%	79%
Carbazole	Questionable Carcinogen			98%	99%
2-Ring PAHs					
Indane				100%	100%
Indene				100%	100%
Naphthalene	Questionable Carcinogen	Yes	Yes	100%	100%
Methylnaphthalene (isomer)				100%	100%
Methylnaphthalene (isomer)				98%	100%
Biphenyl				98%	100%
Dimethylnaphthalene (isomer)	Questionable Carcinogen			100%	100%
3-Ring PAHs					
Acenaphthylene				99%	100%
Acenaphthene				93%	99%
Fluorene		Yes	Yes	95%	96%
Phenanthrene	Questionable Carcinogen	Yes	Yes	87%	97%
Anthracene	Questionable Carcinogen	Yes	Yes	95%	92%
4 & 5 - PAHs					
Fluoranthene	Questionable Carcinogen	Yes	Yes	70%	91%
Pyrene				95%	34%
2,3-Benzofluorene				67%	100%
Chrysene	Confirmed Carcinogen			32%	23%
Benzo[b]fluoranthene	Suspected Carcinogen	Yes	Yes	36%	56%
Benzo[a]pyrene	Confirmed Carcinogen	Yes	Yes	39%	61%
Phenolic Compounds					
Phenol	Questionable Carcinogen	Yes	Yes	98%	98%
O-cresol	Questionable Carcinogen			95%	97%
M-cresol	Questionable Carcinogen			100%	100%
P-cresol	Questionable Carcinogen			100%	100%
xylenol (isomer)				100%	100%
xylenol (isomer)				100%	100%
xylenol (isomer)				91%	94%
trimethylphenol (isomer)				100%	100%
naphthanol (isomer)				92%	96%
pentachlorophenol	Questionable Carcinogen	Yes	No	87%	98%

[a] Groundwater Protection Criteria have been promulgated by the DEP.
[b] Surface Water Protection Criteria have been promulgated by the DEP.

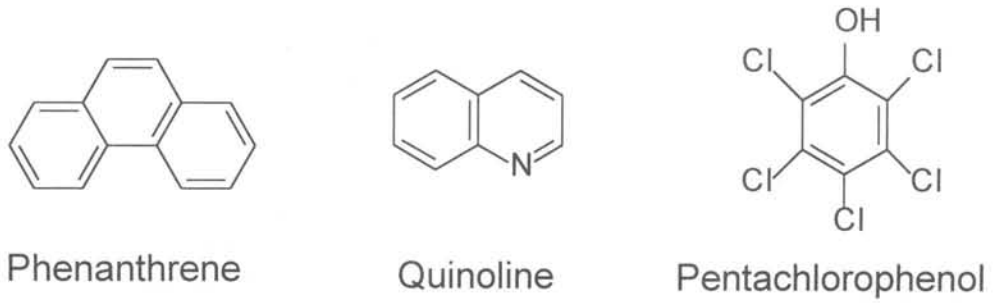

Figure 2. Structure of pentachlorophenol and two organic compounds found in American Creosote-P2.

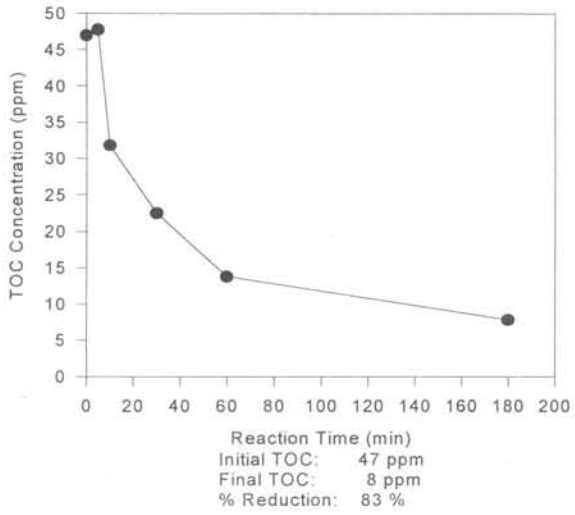

Figure 3. Loss of total organic carbon from solution.

showed that most compounds disappeared—that is, were transformed to other products—within 5 minutes (Table 1).

Total oxidation of the creosote components and pentachlorophenol to carbon dioxide was measured indirectly by monitoring the reaction solution for loss of total organic carbon concentration. The results (Figure 3) show that total organic carbon was reduced from 47 to 8 parts per million over a 3 hour period. Additional experiments showed that all the chlorine in pentachlorophenol was liberated in solution as chloride ion, the main anion in table salt. The inorganic products from nitrogen present in some of the heterocyclic aromatic compounds—nitrate, nitrite and ammonia—were detected in the treated water.

If the creosote components and pentachlorophenol are not completely degraded, their byproducts may exhibit toxic properties. The untreated and the treated solutions were tested for toxicity by the DEP's Water Toxic Laboratory using fathead minnows and *Daphnia* as test organisms. *Daphnia*, commonly known as the water flea, is a freshwater invertebrate that is a source of food for fish and other organisms. Both minnows and *Daphnia* are commonly found in lakes and streams in the Northeast. The toxicity was measured as the LC50, which is the concentration of the toxic substance that will cause death in 50% of the test population. The LC50 is determined from a plot of dose versus response after a 48 hour exposure period. The bioassays indicated that the creosote-pentachlorophenol solution treated by the photo-Fenton process was from ten to fifty times less toxic than the untreated solution for both of these organisms, as shown in Table 2.

We conclude that the photo-Fenton process is effective in degrading pentachlorophenol and creosote/coal-tar compounds and greatly decreases the toxicity of the contaminated water. This process could potentially be used to treat contaminated groundwater.

Table 2. Toxicity to fathead minnow (*Pimephates promelus*) and *Daphnia* (*Daphnia pulex)* of creosote-pentachlorophenol contaminated water before and after photo-Fenton reaction showing 48-hour LC50 (lethal concentration to 50% of the tested population). The number is the fraction of the original solution in the diluted test solution. A larger number indicates that the solution is less toxic for that specific organism. The control consisted of all reagents used except for hydrogen peroxide and creosote-pentachlorophenol.

	Treatment			
Species	None	10 min.	180 min.	control
Minnows	0.01	0.23	0.56	0.71
Daphnia	0.03	0.34	0.55	0.16

Blue mold disease returns to Connecticut to threaten $70 million tobacco crop

By James A. LaMondia and Donald E. Aylor

A disease that had been absent from tobacco in Connecticut for 15 years suddenly reappeared and had devastating effects during 1997. The disease is blue mold, which is caused by a fungal pathogen. Although the extent of crop loss is not fully known at the time of this writing, The Experiment Station helped cope with the disease and lessened its impact.

We were watching for blue mold because it had been in the state before and because it had been gradually building in intensity in the southern production areas. Blue mold disease was found in Connecticut on October 8, 1996. This gave us advance notice and allowed time for us to alert growers and to take actions to lessen the impact of a potential epidemic in 1997.

Blue mold is a devastating foliar disease of both cultivated and wild tobacco (*Nicotiana*) species. This disease is very explosive and is capable of spreading throughout a growing region within a matter of a couple of weeks. The disease is caused by a downy mildew fungus named *Peronospora tabacina*. The fungus is an obligate parasite, meaning that it is obligated to live on the living cells of host plants and can not be cultured *in vitro*.

The blue mold pathogen causes leaf spot symptoms which can result in a complete loss for cigar wrapper leaves such as Connecticut shade and broadleaf tobaccos. Leaves must be blemish free, strong and elastic to produce a high quality binder or wrapper. Diseased leaves are blemished and non-elastic and the off-color lesions become tattered holes in the leaves when they are stretched. Under some conditions the fungus will invade the stems and become systemic, moving rapidly throughout the plant and killing young plants outright,

Blue mold is most severe in wet weather, as the fungus requires leaf wetness and high humidity to infect and produce new spores. The pathogen produces lemon-shaped sporangia (spores) on tree-like branched structures which protrude through the stomata of the leaf. Stomata are natural openings in leaves through which plants exchange carbon dioxide and water vapor. The sporangia are colorless and measure about 20 µm in diameter. They are produced on infected leaves under humid conditions, usually during the evening, and are released into the air to float away in air currents in the morning. The microscopic colorless spores can be blown by wind to neighboring plants or fields, and occasionally they can be carried by wind over long distances to fields in other counties or states. The fungus consistently survives the winter at latitudes south of the 30th parallel (about the latitude of St. Augustine, Florida) and moves northward during the growing season either in transplants or as wind-blown spores. Under some conditions, the fungus can produce thick-walled oospores in the interior of infected leaves. Oospores are sexually produced resting spores and are thought to be tough enough to survive the winter farther to the north.

A single sporangium can regenerate the fungus and, therefore, it acts very much like the seed of a higher plant. Sporangia require high humidity (>98.5%) or free moisture on the leaf to germinate and infect the leaf. Germination and infection can occur in as little as 2 to 4 hours. The fungus hyphal strands quickly invade other cells and grow inside the leaf without visible symptoms and unnoticed for up to 5 to 7 days. It is during this symptom-less (latent) period that the disease can be unwittingly moved from place to place in transplants.

At the end of the latent period the fungus causes leaf spot symptoms consisting of yellow circles about the diameter of a dime to a quarter. Under humid or wet conditions, masses of sporangia are produced on the underside of infected leaves. Millions of spores can be produced on a single infected leaf. These spores are released, usually in early to mid-morning, and move through the air to deposit on tobacco plants and initiate new infections. Exposure of the colorless spores to ultraviolet light in sunshine can kill them within several hours. Because of this and the critical effect of moisture, conditions of high humidity and reduced light levels in shade tents can be especially conducive to disease; dry air and bright sunny skies for extended periods can help slow the progress of the epidemic.

The host range of *Peronospora tabacina* is primarily in the genus *Nicotiana*. Many *Nicotiana* species are susceptible. Plants are usually most easily infected when young and actively growing. Older, mature plants or plants that are flowering are generally not as easily infected. Strains of the pathogen have been identified which differ in their ability to cause disease or sporulate on different plants. Other solanaceous crops such as tomato (*Lycopersicon esculentum*), pepper (*Capsicum annuum*), and eggplant (*Solanum melongena*) have been reported to be resistant or moderately resistant hosts in the United States and Australia. These species are typically not diseased, however, and their role in the survival of the pathogen is probably minimal compared to cultivated tobacco volunteers and wild *Nicotiana* species.

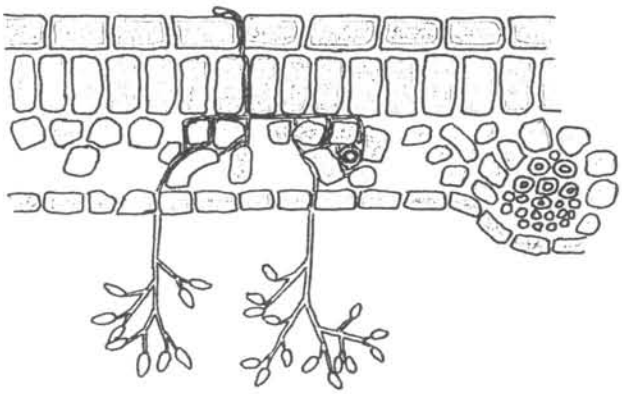

Figure 1. Sporangia of ***Peronospora tabacina*, the causal agent of blue mold. Sporangia are produced mainly on the underside of a tobacco leaf, which is sketched here in cross section.**

The history of blue mold is a story of change. The first published report of blue mold in cultivated tobacco was in Australia in 1890. While the same or a similar fungus was described on wild tobacco species endemic to the North American Southwest, the first report of blue mold on the commercially cultivated *Nicotiana tabacum* in the United states did not occur until 1921 in Florida and Georgia. The disease became destructive and widespread in Florida and Georgia in 1931 and epidemics occurred throughout the southern United States in the 1930's. Blue mold was first found in Connecticut in 1937 in a seedbed in Bloomfield. The disease spread to the field, but soon disappeared in the hot weather of July. From 1937 to 1956, blue mold was commonly found in Connecticut, however, it usually only presented a problem in the seed beds early in the season. Except for very wet years, the disease was rarely seen after plants had been transplanted in the field. From 1958 to 1978, blue mold was either not present or was present only at very low levels that escaped detection.

A severe epidemic of blue mold swept throughout the United States in 1979. The disease was discovered in the field in Connecticut on July 30 and by the end of the season, caused crop losses of approximately 20%. This epidemic was unusual because it occurred in the summer and because the disease had been absent from Connecticut for 15 years. It foretold an apparent change in the fungus that allowed it to thrive in warmer temperatures than in previous years. Moisture still remained the critical factor in the development of disease, so that 2 weeks or more of hot dry weather could halt the epidemic until wet conditions returned.

Another epidemic occurred in 1980, and again losses in Connecticut were significant. That year, blue mold was first confirmed in Connecticut on July 13. Fortunately for growers, a new systemic fungicide called Ridomil (chemical name metalaxyl) with excellent activity against the blue mold fungus was registered in 1980. By 1981, Ridomil was in wide use, and the disease was largely controlled.

However, a cloud was already on the horizon. The first reports of Ridomil-resistant strains of *Peronospora tabacina* were made in 1981 and 1982 in Nicaragua. Resistant strains were reported in Mexico in 1984 and in the United States in 1991. No major disease outbreaks occurred, however, until 1995 and 1996, when strains highly resistant to Ridomil attacked crops throughout southern production areas.

From 1981 until late last year, blue mold was absent from Connecticut (another absence of 15 years). In 1996, blue mold developed such a head of steam in the southern production areas that the epidemic made it all the way to Connecticut by the end of the season. Blue mold was found on October 8, 1996 on a few remaining tobacco plants left standing in the field for seed production. Isolates of the fungus were obtained from several towns in Connecticut. These were tested and found to be highly resistant to Ridomil. Because of the history of back-to-back epidemics in Connecticut we were very concerned about the possibility that Ridomil-resistant blue mold would occur here during the 1997 growing season. To respond to this perceived threat two immediate steps were taken: we encouraged growers to destroy the infected tobacco in 1996 and we applied for an emergency registration for the fungicide Acrobat MZ. This product is a mixture of dimethomorph, a new locally systemic fungicide in the process of registration for tobacco by American Cyanamid Inc., and a protectant fungicide, Dithane, used for many years on a wide variety of crops. Growers were informed of the potential for a blue mold epidemic, encouraged to be on the lookout for disease and encouraged to report potential problems to the Experiment Station. The Acrobat MZ application was approved by The Connecticut Department of Environmental Protection (DEP) and the U.S. Environmental Protection Agency prior to the start of the growing season, and information to this effect was sent to people involved with tobacco.

In 1997, blue mold occurred in nearly all of the tobacco production areas of eastern North America. The first report in commercial tobacco types was in northern Florida on April 23. The disease spread northward from state to state by a combination of wind-blown spores and by the movement in trucks of infected transplants. Throughout 1997, the blue mold epidemic was dominated by Ridomil-resistant strains.

The shipment of blue mold-infected seedlings from North Carolina to Lancaster County, PA resulted in active blue mold in a field there on June 6. This disease outbreak represented a big northward "jump" early in the season, and it may have had a major impact on later disease development in Connecticut. Airborne spores originating from Lancaster County could have reached the Connecticut River Valley in mid June and initiated the 1997 epidemic here. The disease was first reported and confirmed in Connecticut

Tobacco has been grown in the Connecticut River Valley since colonial times. The earliest record of tobacco production in Connecticut was in 1640 in Windsor. By the early 1700's enough tobacco was produced to be an export item, and by the end of that century cigars were being produced. Broadleaf cigar tobacco was developed by the 1840's. This all-purpose field-grown type was capable of producing filler, binder, and wrapper all on the same plant. Shade tobacco was developed at the turn of this century as a high quality cigar-wrapper leaf to compete with the light-colored, thin leaf being grown in Sumatra at the time. The production area of Connecticut cigar tobacco types reached a peak of over 30,000 acres in the early 1920's, before declining with the advent of wrappers made from homogenized and reconstituted tobacco. Tobacco is currently grown in the Connecticut Valley on approximately 3,000 acres. The value of the crop is about $100 million, with a little over $70 million of it being grown in Connecticut.

Over the years, several diseases have seriously threatened the industry. Experiment Station scientists responded quickly to each new threat. Wildfire, a quickly spreading bacterial disease that causes leaf spot epidemics leading to near total crop destruction, was controlled by the development of chemical controls and resistant tobacco varieties in the 1950's. Fleck, a non-parasitic leaf spot caused by cell damage from air pollutants such as ozone, was controlled by the development of resistant tobacco varieties. Fusarium wilt, caused by a soil-inhabiting fungus that infects roots and kills the water conducting tissues in the roots and stem, killed an increasing percentage of the broadleaf tobacco crop until the release of resistant tobacco varieties by the Experiment Station in 1991. The tobacco cyst nematode, a parasitic microscopic roundworm, infects the roots and thereby stunts plant growth and causes drastic reductions in yield and leaf quality. The nematode can currently be controlled by soil fumigation or rotation with non-host crops. The identification and development of resistance to nematodes have been under study for several years. The release of nematode-resistant shade tobacco is imminent.

on July 8. The probable time of first infection was on June 12 or 13. In the weeks following infection, weather conditions were close to ideal for infection, sporulation and disease development. Rain occurred on 33 days out of 75 in the second half of June, July and in August. Heavy dews sufficient to speed the epidemic were present on at least an additional 7 days. The longest interval between infection periods was only 7 days, matching the 5 to 7 day latent period of the fungus very well. The result was an exponential increase in disease and quick spread. The day after we first confirmed the disease in Connecticut, there occurred two days of rain and thunderstorms with the potential to move spores throughout the Valley. One week later the disease had been reported from several new towns and by two weeks it had reached throughout the Valley, as far north as Whately, MA.

The possibility that plant pathogens can be transported hundreds of miles through the atmosphere and initiate new disease outbreaks is real, although there is no direct evidence for this and the evidence favoring long distance dispersal is circumstantial. It has been well documented that pollen, spores, and Saharan dust for that matter, can be carried high into the atmosphere and long distances by wind. Nevertheless, we know relatively little about conditions when plant pathogenic fungus spores can actually travel several hundreds, or thousands, of miles through the air and actually *infect* distant fields. We are working on methods to calculate probabilities of long-distance disease spread, but at present these calculations are very uncertain.

The probability that a *viable* spore, originating from afar, will reach and infect a crop depends on several things, such as air flow from a source of spores to the crop in question, the number of spores released into the air at the source, the dilution of the numbers of spores in the air by the combined effects of transport downwind in a shear flow and atmospheric turbulence, and finally the loss of spores by deposition to the ground and by death during flight.

Given air movement in the right direction to carry spores on a course between a source of spores and our crop, there are three major factors affecting the chance of disease transport: 1) spore production at the source, 2) dilution of the spore cloud and 3) mortality of spores during transport. The fungus *P. tabacina* is a prolific producer of spores: A 500 ha area of heavily diseased tobacco can produce about 64 trillion (6.4×10^{13}) spores per day. During a journey of 20 to 30 hours (which is about the time it can take for air to be transported 300 to 450 miles in the atmosphere), the concentration of spores can be reduced by atmospheric diffusion and shear dispersion in the absence of rain by about a factor of 10^{13}. Comparison of these numbers suggests that some spores will make it through, but will they be dead or alive? In full sunlight, 99% of *P. tabacina* sporangia in the air are killed in about 6 hours. In full overcast (complete cloud cover) conditions, however, 80% of the sporangia in the air survive for 6 hours or more. Survival is the key to establishment of disease at long distance. Clearly, spores have a much better chance if they travel under cloud cover. Thus, depending on sky conditions during transport, there may be considerably more (sunny) or comparable (cloudy) danger to a tobacco field from a small, potentially unnoticed, local source than from a massive source 450 miles away.

In 1997 virtually all growers saw some disease, and damage ranged from light to very extensive. Despite the fact that over 90% of the tobacco crop was still in the field and vulnerable when the disease was confirmed and that ideal

weather conditions for disease development prevailed throughout the summer, much of the crop was still harvested. The harvest that was salvaged was largely a result of the many timely actions taken by Experiment Station scientists, DEP regulators, chemical companies and growers. As soon as blue mold was confirmed, we began greenhouse tests to confirm Ridomil resistance, called growers, buyers and suppliers to spread the warning, alerted DEP, and contacted agricultural chemical companies about support for emergency registration for additional fungicides. Because Acrobat fungicide could not be used closer than 30 days to harvest, growers needed additional fungicides with shorter pre-harvest intervals. Dithane was registered with 1 day to harvest and Aliette was registered with 14 days to harvest. Both fungicides are commonly used on a number of food crops with short days to harvest intervals. Later in the season, DEP declared a crisis exemption for Aliette allowing its use within 3 days to harvest.

As we look ahead and anticipate potential continued outbreaks of blue mold in 1998 and beyond, we are undertaking new research projects to address blue mold. Different sources of tobacco resistance, either recovered from wild *Nicotiana* species or developed as chemically-induced mutants, will be crossed with Connecticut tobacco types as a possible long-term blue mold control tactic. The possibility of local survival of the pathogen over the winter has been controversial. Attempts to determine whether the fungus can be carried to new plantings via infected debris associated with the seed and whether it can survive over the winter in infected stalks are under way. We have also tested a number of ornamental tobacco species for susceptibility to blue mold and determined that *Nicotiana alata*, *N. sylvestris* and *N. sanderae* are all susceptible at the seedling stage. Importantly, we observed that plants infected with Tobacco Mosaic Virus were free of blue mold in field plots with a high incidence of disease. We will evaluate the effect of mild strains of the virus on plant growth, virus symptom development, if any, and on protection from blue mold. By taking these steps it is hoped that the impact of blue mold can be greatly curtailed.

The Connecticut Agricultural
Experiment Station
P.O. Box 1106
New Haven, CT 06504-1106