

# Frontiers of Plant Science

A REPORT FROM THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION, NEW HAVEN

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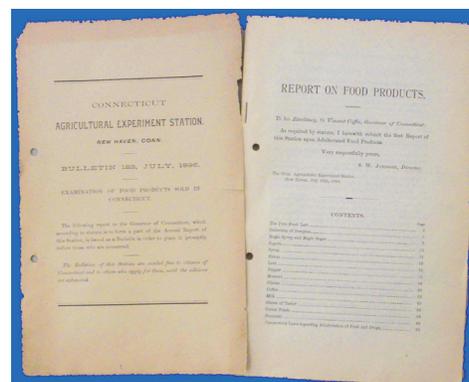
By Dr. David E. Stilwell<sup>1</sup>, Craig L. Musante<sup>2</sup> and Dr. Brij L. Sawhney<sup>3</sup>,  
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Effects of coating CCA pressure treated wood

### Food Analysis at The Connecticut Agricultural Experiment Station

Dr. Walter J. Krol, Department of Analytical Chemistry, The Connecticut Agricultural Experiment Station.



The first report made on the examination of food products sold in Connecticut, 1896

### Protecting the Food Supply and More: New Initiatives in Analytical Chemistry

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New GC/MS system in Analytical Chemistry



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# Effects of Coating CCA Pressure Treated Wood on Arsenic Levels in Plants and Soil

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For many years, chromated copper arsenate (CCA) was the predominant formulation used in the pressure treatment process to preserve wood from decay and insect damage. However, a number of investigations conducted here at The Connecticut Agricultural Experiment Station and elsewhere, showed that varying amounts of CCA were dispersed from the wood by leaching, erosion, weathering, decay and physical dislodgement. The potential environmental problems associated with this dispersal resulted in a phase out of its use in the US for most residential applications effective January 2004. Nonetheless, CCA wood produced prior to the phase out is expected to remain in service for many years, and its use is still permitted for many applications outside the residential setting, such as utility poles and docks.

One major application for this wood was in situations involving soil contact in and around gardens, such as raised-bed gardens, and along fences. In this context, there are health concerns related to arsenic (As) exposure by physical contact with the wood, by contact with soil elevated in arsenic due to leaching, and by arsenic uptake by edible plants grown near CCA preserved wood.

A promising treatment for minimizing CCA dispersal is to coat the wood with a paint, stain, sealer or varnish, thus forming a barrier between the wood and the environment. We tested the use of coatings to prevent preservative dispersal from CCA wood in a soil environment, by coating boxes made from this wood with both film forming (FF) and penetrating finishes (PF), filling them with soil, and weathering them for 2 years. During this time, the soil was sampled periodically and after 2 years, plant uptake of arsenic was determined by growing romaine lettuce, arugula, basil and chives in these boxes under greenhouse conditions.

Each paint or stain was applied in two coats. As shown in Table 1, the coatings consisted of oil-based, semi-transparent stains (two brands, ② one with and the ③ other without alkyd resin ingredients), ④ water based coatings (two brands, ⑤ one with a penetrating alkyd/ acrylic formulation), an ⑥ acrylic solid color deck stain, and a ⑦ polyurethane enamel. Two of the boxes made from CCA wood were left uncoated, as was the control box.

Coating/Box #	Coating*	Base	Color	Cover
1	None			
2	Sealant with Alkyd and Acrylics	Water	Clear	Clear
3	Deck and Siding Stain	Oil	Gray	Semi
4	Sealant	Oil	Clear	Clear
5	Deck Stain with Alkyd Resin	Oil	Gray	Semi
6	Solid Color Acrylic Deck Stain Polyurethane Floor and Deck	Water	White	Opaque
7	Enamel	Oil	Gray	Opaque
8	None			
9	Untreated Pine			

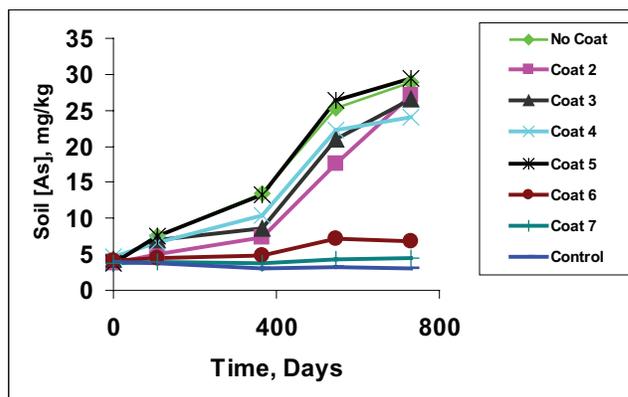
**Table 1. Description of Coatings.** \* *Brand and Code: Coating 2, Behr, 300; 3, Behr 1-765; 4, Thompson's; 5, Olympic, 53178; 6, Olympic, 53097; 7, Sapolin, 40-9309.*

The boxes were filled with a mixture of 90% soil (sandy loam) and 10% compost (by volume) and placed out to weather (Figure 1). The soil was sampled periodically using a 2.2 cm diameter soil corer, 0-3 cm from the wood to the box bottom, taking one sample from each of the four sides. After weathering for two years, samples were taken 6 cm from the edge and from the center of each box. Elemental analysis of the soil, plant and wood samples was determined, following nitric acid digestion, using a Thermo Jarrell Ash ICP-AES Atom Scan 16 atomic spectrometer, or in samples containing low arsenic (<0.1 mg/l in solution) the more sensitive technique of graphite furnace atomic absorption (GFAA) was employed using a Perkin Elmer 5100 instrument.



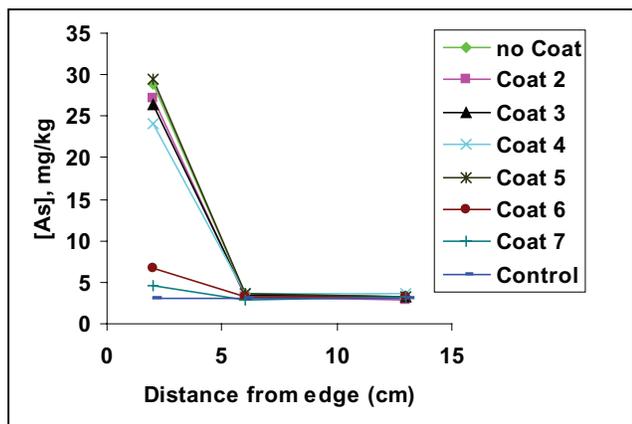
**Figure 1. Coated Boxes prior to weathering.**

The average soil arsenic levels next to the wood over time for different treatments are given in Figure 2. Arsenic levels in the soil samples from the uncoated CCA boxes increased with time of weathering. Furthermore, the average arsenic level in soil samples taken from the uncoated boxes, after 365 days of weathering, exceeded the State of Connecticut limit of 10 mg/kg. The lowest soil As levels were from boxes coated with opaque finishes. These levels,  $6.8 \pm 0.6$  (coating 6) and  $4.6 \pm 1.5$  (coating 7) mg/kg As, though elevated with respect to the As in soils from the control box ( $3.0 \pm 0.2$  mg/kg) maintained a level below the 10 mg/kg State of Connecticut limit throughout the 2-year period. In terms of percent reduction in soil arsenic, the opaque acrylic finish (#6) reduced the arsenic level by about 80%, while the polyurethane based finish (#7) was around 95% effective over the entire 2-year time. Opaque finishes were also found by us to be the most effective coating to reduce arsenic dislodged from surfaces. Although the penetrating finishes (PF) (#2-5) reduced the arsenic levels initially by up to 60%, the effectiveness deteriorated after 1.5 years of weathering, and in fact, after two years of weathering, the soil arsenic was not significantly different from the soil in the uncoated CCA boxes.



**Figure 2. Comparison of soil arsenic versus time for different wood coatings (See Table 1).**

Shown in Figure 3 is the relationship between soil As and distance from the box edge. The concentrations of As in soil 6 cm away from the edge of the boxes are significantly less than the levels in soil next to wood. The average arsenic in all samples 6 cm from the box edge were at most 0.7 mg/kg higher than the average of  $3.1 \pm 0.2$  mg/kg in the control soils samples taken at this time. At the box center (13 cm), As levels in all treatments were within 0.3 mg/kg of the control except for Box 4 sample which was 0.6 mg/kg higher. Thus, beyond 6 cm from the edge of the wood the soil arsenic levels are reduced to well within 1 mg/kg of background levels of 3-4 mg/kg in this type of soil. This immobilization of As by the soil is likely due to the presence of iron compounds and clay minerals which are known to fix As. Lateral decreases in soil As, reaching background levels within 15-130 cm from the CCA wood, also has been observed by us in a study on arsenic levels in soils near traffic sound barriers built with CCA wood.



**Figure 3. Soil arsenic with distance from box edge, after weathering for two years.**

After two years of weathering, arugula (*Eruca sativa*, rocket), romaine lettuce (*Lactuca sativa*), sweet basil (*Ocimum basilicum*), and chives (*Allium schoenoprasum*) were grown in these boxes in a greenhouse. The seeds were germinated in 1.2x1.2x2.6 plugs in a starter tray filled with growth media. After germination and sprouting (14 days lettuce and arugula, 21 days chives and basil), the seedlings were transplanted into the box soil (Figure 4). The arugula seedlings were planted, equally spaced, 2 cm from the box edge, 4 along one side and 3 along an adjacent side. Two seedlings were also planted, equally spaced, 8 cm from the edge of the two sides and one seedling was placed in the center of the box. The lettuce seedlings were planted similarly along the remaining two sides, 3 seedlings 2 cm from the edge per side, and one seedling on each side, 8 cm from the edge. The chives and basil were planted in the corners of each box and with each type on opposite sides. Water was supplied as needed, typically 1 liter per box every other day. On seven occasions fertilizer was added to the water at a rate of 30 mg/l N/P/K. Over the growing period approximately 300 mg of P was added to the approximately 10 kg of soil.



**Figure 4. Plants in Boxes 16 days after transplant.**

The entire plant was harvested after 21 days of growth for the lettuce and arugula, and after 28 days for the chives and basil, by cutting them off within 1 cm of the soil line. All of the plants were rinsed with distilled water, dried at 80°C for 10 hours in paper bags, crushed and transferred to polypropylene containers. After acid digestion in concentrated nitric acid, the arsenic in the plant tissue was determined by atomic spectroscopy as described above.

The amounts of As in the plants and soil at the box edges, with finish type, are shown in Table 2. The average amount of As in the soil next to the box edges was 29±7 mg/kg next to uncoated CCA wood, 27±6 next to wood with PF coatings and only 6±2 next to wood with FF finishes. Average amounts of As (mg/kg, dry weight) in arugula grown 2 cm from the CCA wood was 60±0.1 (4 composites), 61±13 (8 composites) from wood coated with PF finishes and 24±7 (4 composites) in those coated with FF finishes. Similarly, the amounts in chives were, 75 (CCA), 75 (PF), 12 (FF); lettuce 5 (CCA), 5 (PF), 1.4 (FF); basil 6 (CCA), 10 (PF), 3 (FF). The amounts of As in plants grown in the control boxes were all <1 mg/kg. Clearly, there was no reduction in plant As when plants were grown next to the non-opaque finished wood, while the reduction in plant As ranged from 50-84% in plants grown next to the opaque finished wood. The amounts of arsenic in the arugula and chives grown in the CCA boxes exceeded the British limit for plant As of 1 mg/kg on a fresh weight basis (10-14 mg/kg dry weight basis).

Finish	Soil	Arugula	Chives	Basil	Lettuce
None	29 ± 7	60 ± 0.1	75 ± 19	6 ± 2	4.9 ± 0.6
Penetrating	27 ± 6	61 ± 13	75 ± 24	10 ± 3	4.8 ± 0.5
Film Forming	5.7 ± 1.6	24 ± 7	12 ± 3	3 ± 0.3	1.4 ± .25
Control Wood	3.0 ± 0.2	0.5 ± 0.2	<0.2	0.9 ± 0.7	0.2 ± 0.01

**Table 2. Arsenic (mg/kg, dry weight basis) in soil and plants next to CCA and control wood boxes coated with different finishes.**

The amounts of As in the soil and in plants grown with distance from the edge of the box and type of finish are shown in Figure 5. Although the plant As followed the trends in soil As, and the amounts of As in plants grown 6 cm from the box edge compared to 2 cm from the edge were lowered by 55 to 84%, these amounts were well above the background levels in plants grown in the control soil. Furthermore, the As levels in arugula plants grown in the box center (13 cm from the edges) did not decrease significantly

from the levels in plants grown 6 cm from the edge. Also, the As in the arugula plants grown 13 cm from the edge in the CCA boxes, ranging from 7 to 18 mg/kg, was significantly above the 0.5 mg/kg As levels in arugula plants grown in the control boxes, even though the soil As in the center of the box was at or near background. This increase in uptake of As in the plants probably results from root growth into areas of As contamination, and by the continuous leaching of a fresh supply of As from the wood.

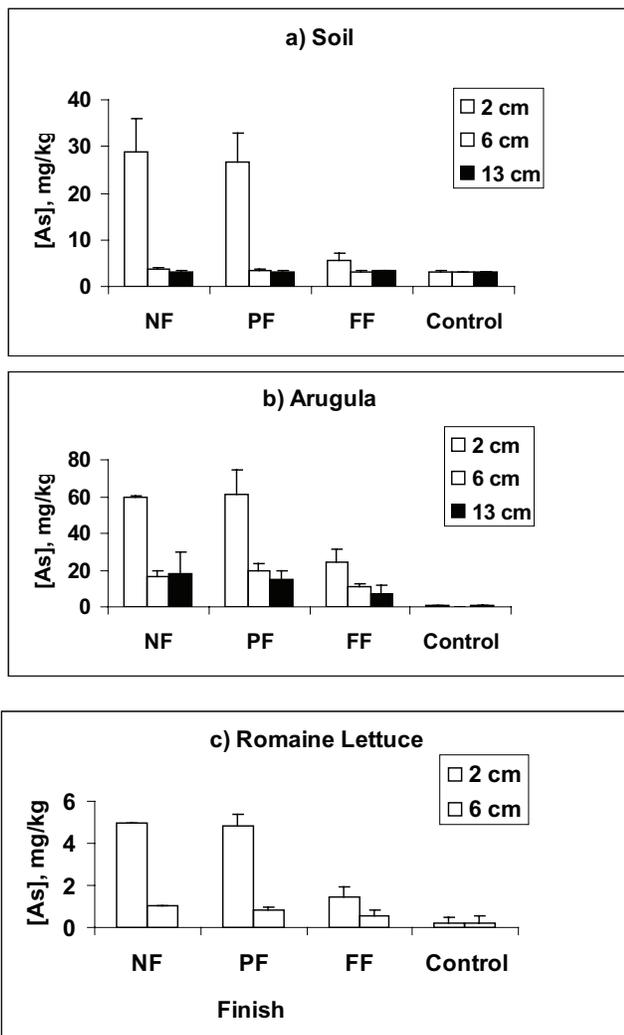


Figure 6. Soil and plant As with distance from edge of box and type of finish. a) Soil, b) Arugula, c) Romaine Lettuce (NF= No Finish, PF= Penetrating finish, FF= Film forming finish).

## Conclusions

Over the two-year weathering period, the As levels in soils within 2 cm of the uncoated CCA wood increased from  $3.7 \pm 0.1$  to  $29 \pm 7$  mg/kg. Moreover, within one year of weathering, the arsenic next to uncoated CCA wood increased to levels that not only exceeded the State of Connecticut limit of 10 mg/kg, but which were also on the upper bounds of As limits (2-26 mg/kg) set by other local, state and federal government agencies. This contamination, however, appears to be localized to soil within a few cm of the CCA wood. Soil samples, taken 6 and 13 cm from the box edge after two years of weathering, were at, or near background levels for As.

Opaque coatings formulated using acrylics or polyurethane when applied to CCA wood reduced the migration of arsenic from the wood into the surrounding soil by 80% to 95%, which kept the As levels in the soil below the regulatory limit over the entire two year

weathering period. Other coatings, either oil or water based, but with clear or semi-transparent coverage, while initially reducing the arsenic migration up to 60%, did not appear to exhibit any protective properties after two years of weathering. Clearly, the film forming opaque finishes are effective in reducing leaching and dislodgeable arsenic from CCA treated wood. The penetrating semi-transparent and transparent finishes, though useful in above ground situations, proved to be very limited when used in contact with soil.

The plant uptake of As followed the order Chives > Arugula > Basil > Lettuce. Compared to plants grown next to uncoated CCA wood, there was no reduction in plant As when grown along the edge of CCA wood coated with penetrating finishes, while in plants grown next to opaque finished wood the reduction in plant As ranged from 50-84%. The As reduction in plants grown 6 cm from the wood compared to 2 cm from the wood ranged from 55-84%. The amounts of arsenic in the arugula and chives were significant and exceeded the British limit for As in edible plants of 1 mg/kg, fresh weight basis (10-14 mg/kg, dry weight basis). The As in the basil was near the limit and the lettuce plants were all below the limit.

## Suggested Reading

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**Dr. David E. Stilwell** was born in Aberdeen, MD and raised in Mt. View, CA. After working in the semiconductor electronics industry for some time, he attended the University of New Mexico in Albuquerque, where he received a Bachelor degree in Chemistry in 1980 and a Ph.D. in Analytical Chemistry in 1987. Following a postdoctoral fellowship with the Office of Naval Technologies, China Lake CA, and a brief teaching position at Southern Oregon State College in Ashland OR, he joined the Station Staff in 1990. Dave's interests are primarily focused on the study of processes that affect the amounts and movements of metals in the environment, and he is particularly active on work on the environmental effects on the use of wood preservatives containing copper, chromium and arsenic (CCA), and on lead in community garden soils. He is also involved in the analysis of feeds and fertilizers conducted to confirm label guarantees, as well as on the analysis of food, toys and animal samples for contamination by heavy metals.

**Mr. Craig L. Musante** received his Bachelors of Science degree in Chemistry in 1988 from Sacred Heart University and his Masters degree in 1992 from The University on New Haven. He is currently employed at The Connecticut Agricultural Experiment Station as a research technician II in the Department of Analytical Chemistry. Mr. Musante's past responsibilities included the routine elemental analysis of materials, such as fertilizers, feeds, soils, water, and plants, using atomic spectroscopy (ICP-AES, and GFAA). His research has focused on heavy metals and arsenic in the environment. Currently, Mr. Musante assists Dr. David Stilwell in his studies of copper, chromium, and arsenic (CCA) containing wood preservatives and their impact on the environment. Mr. Musante's current responsibilities consist of the elemental analysis of foods using ICP-MS for the FDA's FERN program.

**Dr. Brij L. Sawhney** received his B.S. and M.S. from Panjab University, India in 1951 and 1953 respectfully, followed by receiving his Ph.D. from the University of Wisconsin in 1958. Dr. Sawhney was hired as an Assistant Soil Scientist in 1962, promoted to Associate Soil Scientist in 1964, then to Soil Scientist in 1967, retired in 1997 and was honored with emeritus status in 1997. Dr. Sawhney's past research was on the formation and weathering of soil material and their interactions with radioactive pollutants. His current research is with the reactions and movement of inorganic and organic pollutants in soils and groundwater.

# Food Analysis at The Connecticut Agricultural Experiment Station

Dr. Walter J. Krol, Department of Analytical Chemistry, The Connecticut Agricultural Experiment Station.

## Abstract

The Connecticut Agricultural Experiment Station (CAES) has been involved in the analysis of food for adulteration since its inception in 1875, and has published, at least in part, an annual report of its findings on a yearly basis for the past 130 years. The earliest studies focused on the dilution of food products with filler materials, thereby increasing profit for the merchant. Following World War II, the invention of man-made pesticides and their intentional application to food products concentrated efforts on the determination of their residues in food to ensure that the consumer would not be injured as a consequence of their use. Today, working in collaboration with the Food and Drug Administration (FDA) and United States Department of Agriculture (USDA), the Department of Analytical Chemistry at the CAES is one of eight Co-Operative Agreement Program (CAP) laboratories in the country providing surge capacity to these agencies in the event of chemical terrorism against the nation's food supply.

## Introduction

According to USDA records, elemental sulfur combined with tobacco leaf extract was the first agent to be used as a pesticide in the US in 1854 for the control of sheep scab. Through the latter half of the 19<sup>th</sup> century farmers turned to naturally occurring materials to protect their crops from damage arising from insects and disease. Although a very poisonous plant, California false hellebore (*Veratrum californicum*) was often employed medicinally by a number of native North American Indian tribes who used it mainly as an external application to treat wounds. The dried and powdered root was used as an insecticide and a parasiticide. It was also effective against caterpillars and mammals.

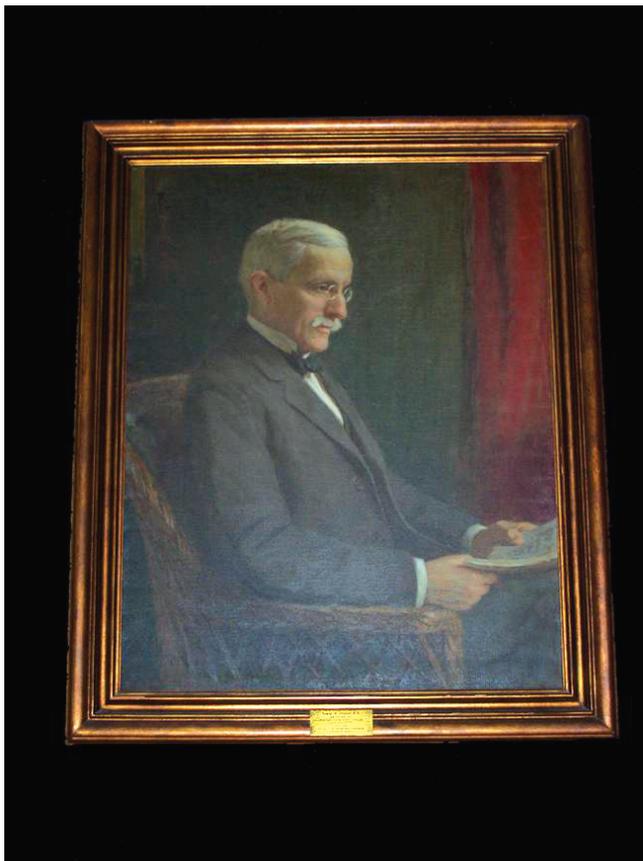
In the 1860s, various byproducts of the chemical dye industry were found to have insecticidal properties. With exotic-sounding names like Paris green and London purple, these substances became increasingly popular. Their common ingredient was arsenic; arsenic-based compounds were popular during the Victorian era as pigments for candles and wallpaper, as cosmetics and patent medicines; only later were the lethal properties of various arsenic derivatives more widely recognized. Lead arsenate was overwhelmingly the most popular insecticide throughout the early 20<sup>th</sup> century; it often killed plants, bees, and livestock as readily as it killed pestiferous insects. Still its popularity remained uncontested for several decades. There was a so-called *Fliegenstein*; a condensed white arsenic that killed flies that landed on it, and an arsenic soap for preventing the growth of larvae in animal hides.

Pyrethrum, composed of pyrethrins, is an extract of the pyrethrum flower of the *Chrysanthemum* family, largely *Chrysanthemum cinerariaefolium*. Extracts were produced in California beginning around 1871, and widely used for their insecticidal properties. The extracts contain 20-50% total pyrethrins, the main active constituents being pyrethrin I (P1) and pyrethrin II (P2), with smaller amounts of related cinerins and jasmolins and are still widely used today. The 1870's and 80's also saw the use of oil, and its petroleum byproducts for the control of insects and weeds. The use of highly toxic hydrocyanic gas (HCN), later known infamously as Zyklon-B, gained popular use in 1886 as a household fumigant and was used as such into the 1920's. For a more comprehensive list of chronological history of the development of insecticides and control equipment from 1854 through 1954 the reader is referred to the Clemson University website: (<http://entweb.clemson.edu/pesticide/history.htm>).

There was no doubt that the use of these early pesticides to control insect infestations and disease was beneficial to the farmer. They were readily available, relatively inexpensive, provided increased yield, and perhaps most importantly, their use was unregulated. Worker and consumer safety, as well as environmental concerns, were ancillary issues. By the turn of the 20<sup>th</sup> century there seemed dim hope that the Federal Government would become involved to correct these issues due to Congressional deadlock.

## Samuel William Johnson (1830 – 1906)

Samuel William Johnson was born in Kingsboro, New York July 3, 1830, with an ancestry that traces back to the early Connecticut settlers. Johnson's childhood was spent on a farm where he acquired an interest in the scientific side of the processes of plant and animal life, and the dignity of agriculture. He wrote his first paper "Fixing ammonia" at the age of 17, then studied at Yale College under J.P. Norton before embarking on his European studies in Germany, France and England where he gained experience in inorganic analysis and organic chemistry and its relation to plants and animals under the direction of Professors Erdmann, Neumann, Liebig, and Farnakland. During his studies, he visited the experimental farms of Lawes and Gilbert, and numerous other sites of agricultural interest. During his travels, he became friends with Evan Pugh who was then studying the same curriculum with the same objectives in mind as himself. Pugh later became head of the College of Agriculture of Pennsylvania.



**Portrait of Samuel Johnson on display in the Johnson-Horsfall building at the CAES.**

Upon his return to the United States, Johnson joined the teaching staff at Yale and within two years was appointed Professor of Analytical and Agricultural Chemistry in the Yale Scientific School. He then set himself to work to develop a popular interest in scientific agriculture. Through his popular essays and lectures throughout New England and New York he conveyed the science of agriculture to the layman in layman terms. In 1856, he was appointed chemist to the Connecticut State Agricultural Society, and in 1857, produced the first scientific report of its kind in the country. *He placed a monetary value on commercial fertilizer.* The report was more than a chief chemist's statement of analytical results; it instructed the farmer of the great practical importance to the conduct of his business. This report was *The Beginning* of the agricultural movement in the US which led to the establishment of an agricultural experiment station in every state.

Following the Civil War, Johnson devoted himself to practical questions rather than those of purely scientific interest, and working with the Connecticut Agricultural

Society, established a Board of Agriculture. His petitions to the state to establish a workplace to furnish the farmer with scientific information and investigations as he required for the successful conduct of his farm, met success in 1875 when the state appropriated \$2,800 annually for two years of investigations to be carried out in the laboratories of Wesleyan University under the direction of W.O. Atwater. Wesleyan provided the needed laboratories free of charge.

Two years later, in 1877, through the direct efforts of Professor Johnson, the First Agricultural Experiment Station of the United States was formed on a permanent basis, and the state legislature incorporated the Connecticut Agricultural Experiment Station as an independent institution, under the management of a board of control representing the agricultural and scientific interest of the state. Professor Johnson was at once appointed the director, and remained in this position for 23 years (1877 – 1900) when he resigned. His work succeeded in demonstrating in a practical manner what experimentation could do for the agricultural community.

In his 1910 report, the President of Yale University wrote of Johnson: *It has been said that the most substantial contribution of the United States to applied science has been in using chemistry for the improvement of agriculture. Of this movement Professor Johnson was the leader. The whole system of the agricultural experiment stations may well be regarded as his monument.*”

### **Early Food Chemistry**

In return for the funds provided by the State, in January 1895 a Public Act entitled “An Act Regulating the Manufacture and Sale of Food Products” was passed: Section 4 of this act states that “*The Connecticut Agricultural Experiment Station shall make analysis of food products on sale in Connecticut suspected of being adulterated, at such times and places and to such extent...may take from any person...any article suspected of being adulterated...and the said station may adopt or fix standards of purity, quality, or strength when such standards are not specified or fixed by Statutes...the said station shall make an annual report to the Governor upon adulterated food products, in addition to the reports required by law... and said report may be included in the report which said station is already authorized by law to make, and such annual reports Shall be submitted to the general assembly at its regular session...*” CGS, Chapter CCXXXV, 1895.

This task initially seemed monumental, but owing to the lack of Federal Laws Professor Johnson began analyzing various food products for adulteration. In 1895 he and his colleagues analyzed 848 samples of food products from 14 categories. Of the 61 samples of maple sugar, and 41 samples of honey analyzed, 8 samples of maple sugar and 5 samples of honey were found to be adulterated by the addition of sugar. The state of coffee proved far worse. Of the 64 samples analyzed, 58 were found to be adulterated by the addition of roots of chicory, dandelion, and the grains wheat, barley and other legumes. Of the samples analyzed that first year, 67.2% were pure, 29.9% were adulterated, and 2.9% were doubtful.

Between 1896 and 1930, nearly 50,600 food samples were analyzed by personnel at the CAES. It was during this time that different chemical analyses were applied to different types of food to determine if they were pure, or if they had been adulterated. Olive oil, for example, might be diluted with cottonseed oil. The oil in question was tested to determine if such adulteration had occurred through the use of the *Halphen* test for cottonseed oil (red coloration upon heating in carbon disulfide with amyl alcohol and sulfur). Numerous other test procedures, each specific for a single oil type, were undertaken on each oil sample to judge whether adulteration had occurred. These analyses were notably very labor intensive and time consuming. Additional investigations were also made for preservatives, such as sodium benzoate and oxalic acid which was added to food to prolong its shelf life, though at the time little was known about how humans would tolerate these additives. Studies were also made on drug products available for sale at the time.

### **Man-Made Pesticide Residues and Their Analysis**

The discovery and use of the man-made chemical DDT in 1942, shortly after the passage of the Federal Food, Drug, and Cosmetic Act in 1938, changed the way that we would look upon food adulteration to this day. The chemical rapidly began showing up in the food supply, and in those consuming the food. Moreover, in rapid succession, numerous man-made chemicals found use in agriculture, with their residues finding their way to dinner tables across America and the world.

To determine DDT in foods, a colorimetric procedure known as the *Schechter-Hailer* test was developed, which was highly specific for DDT and its metabolites. These tests, and others like it, were used by personnel at the CAES for the determination of the new chemical pesticides which might adulterate food. As in the previous testing, these tests also suffered from the fact that they were *very* labor intensive, time consuming, and many such tests were required for each food sample analyzed. To highlight these points, one needs only to recall the aminotriazole cranberry scare of 1959, which led Congress to pass the Delaney Clause.

In early November 1959, relatively high residues of the herbicide aminotriazole were found in samples of cranberries by the FDA. At the time the material was known as a carcinogen, and little was known about how much human consumption would lead to cancer. The personnel at the CAES worked around the clock for 10 days prior to Thanksgiving to perform analysis on *all* samples of cranberries being sold within Connecticut. On the day before Thanksgiving, the staff reported that all cranberries sold within Connecticut were safe for consumption.

In the early 1960's, the commercial availability of the Gas Chromatograph (GC) revolutionized the way in which foods could be analyzed for pesticide residues. Each food sample could now be extracted, and the extract separated and analyzed simultaneously for several different pesticides which might be present. This initiated a more modern way for the analysis of food adulteration, and following the analysis of 28 samples consisting of 22 apples, two cabbages, three cauliflowers and one strawberry in 1963, none of which was found to contain residues, a new pesticide residue program at the CAES was begun.



**CAES chemists in 1963 using an early gas chromatograph.**

From 1963 through 1993, the detection of pesticide residues in food extracts was performed by GC employing various highly sensitive detection devices, each individually specific to certain elements contained within the pesticide being analyzed. Initially the halogenated pesticides could be determined by employing a hydrogen flame detector. This served well for the detection of the early generation of pesticides most of which were highly halogenated. In 1965, a sodium Thermionic Emission Detector (TED) was built in our laboratories and used for the detection of those insecticides containing phosphorous.

These early detectors, of relatively low sensitivity, gave way to newer, more specialized detectors in the 1970's and 1980's. Detectors such as the Nitrogen Phosphorous Detector (NPD), and the Hall Electrolytic Conductivity Detector (ELCD) specific and highly sensitive for nitrogen, phosphorous, sulfur and halogenated pesticides were routinely employed in our labs. They are, however, no longer used in our work. All of these detection devices, while extremely sensitive and accurate, had a single major drawback: they could not unequivocally identify the pesticide being detected.

In the late 1980's and early 1990's, it became economically and technically feasible to couple the GC separation with Mass Spectral Detection (MSD). This method for the first time allowed the unequivocal identification and quantification of each specific pesticide residue found. It is known that each chemical or pesticide possesses its own unique mass spectral 'fingerprint' which allows its identity to be confirmed. This is analogous to our own fingerprints or DNA, unique to each individual. Since 1993, we have routinely confirmed pesticides found with other detectors such as the NPD and ELCD by taking its chemical 'fingerprint' with a MSD. Employing newer instrumentation purchased in 1999, all pesticide residues in food extracts are analyzed simultaneously by Electron Capture Detection (ECD), specific for halogenated and aromatic chemicals, and MSD to confirm their identity.



**A modern GC/MS instrument with an auto sampler used in pesticide residue analysis.**

In 2005, the use of a High Pressure Liquid Chromatograph (HPLC) in tandem with the purchase of a new Mass Spectrometer (MS), collectively known as HPLC/MS, increased our ability to detect different types of pesticides and obtain their chemical 'fingerprints' by using new and different methodology. It has also lowered our detection limits into the parts per trillion (ppt) realm. In 2006,



**A modern HPLC/MS instrument used for trace level pesticide residue analysis.**

we have refined our existing methodology used for the extraction of pesticide residues from sample matrices, making the extract amenable for analysis employing this new instrumentation in combination with other new GC instrumentation acquired in 2005.

Beginning with the inception of our market basket study in 1963, Connecticut is the only state in New England that has continuously monitored its food supply for pesticide residues in cooperation with the Connecticut Department of Consumer Protection (DCP). Food commodities included in all these studies were not only grown in Connecticut, but also in other parts of the world. The results of the pesticide monitoring study have been published, at least in part, on an annual basis since 1963 and in a bulletin of the present form since 1988.

From 1990 through 2005 using 'modern' instrumentation, an average of 302 samples per year (4846 total samples) were analyzed specifically for pesticide residues as part of our market basket survey. Of these, 64 percent (3103 samples) contained no detectable pesticide residues. A total of 1675 (34.6%) contained at least one pesticide residue below the allowable tolerance (level) set by the Environmental Protection Agency (EPA). The remaining 1.4% of samples was found to be in violation of EPA tolerances. Nine (0.2%) were found to have residues above the EPA tolerance, and 59 (1.2%) were found to be no tolerance violations, either intentionally or unintentionally applied to crops on which the pesticide chemical is not allowed. The results of our findings are published annually, and may be found on the CAES website: <http://www.ct.gov/caes/cwp/view.asp?a=2826&q=378142>. These bulletins compare the findings of the CAES survey with those of the FDA and contain additional useful information for the concerned consumer.

**Dr. Walter J. Krol** was born in Wallingford, Connecticut and attended the University of Connecticut at Storrs, where he received a Bachelor's degree in Chemistry in the spring of 1984. He matriculated to the Johns Hopkins University in Baltimore, Maryland, and, working under the tutelage of Professor Craig Townsend, received his Ph.D. in Bioorganic Chemistry in 1991. He studied as a post-doctoral research fellow for two years' under the direction of Professor Samuel Danishefsky at the Memorial Sloan-Kettering Institute in New York City. In 1993, he joined the American Cyanamid Company in Princeton, New Jersey where he worked for five years' as a tracer synthesis chemist. In 1998, he joined the staff at The Connecticut Agricultural Experiment Station in New Haven as an Assistant Agricultural Scientist II in the Department of Analytical Chemistry.

Walter has studied the biosynthetic origin of the  $\beta$ -lactamase inhibitor clavulanic acid by *S. clavuligerus* using isotopically labeled intermediates. His studies in New York on the total synthesis of the chemotherapeutic drug Paclitaxel (Taxol) led to two patents and culminated in its total synthesis in 1996. At American Cyanamid, Walter synthesized isotopically labeled pesticides to support metabolism, residue and environmental studies required by the EPA for their registration. During the course of this work, he isolated isotopically labeled nemadectin from cultures of *Streptomyces cyaneogriseus sp. Noncyanogenus*, which was used to synthesize carbon-14 labeled moxidectin. This was being developed as a highly efficient ectoparasitic anthelmintic. His current work efforts focus on pesticide residues in food and in the environment. Yearly bulletins published on the results of this work can be found on the CAES website: [www.ct.gov/caes](http://www.ct.gov/caes).

## Protecting the Food Supply and More: New Initiatives in Analytical Chemistry

Dr. MaryJane Incorvia Mattina<sup>1</sup> and staff, <sup>1</sup>Head, Department of Analytical Chemistry,  
The Connecticut Agricultural Experiment Station.

When the Connecticut Agricultural Experiment Station was chartered in 1875, the United States was largely an agrarian society. The initial functions of the institution were to provide support to the farmers and growers of the State through the analysis of fertilizers and animal feeds. As our society has evolved, so have the efforts of the staff of the Station. However, the institution's mission remains focused on "Putting Science to Work for Society" through research, service, and outreach activities.

In the Department of Analytical Chemistry, we continue to perform State-mandated analyses of feeds and fertilizers, while at the same time assuring that our activities remain relevant to the present needs of Connecticut's citizens. In recent decades, those activities with explicit impact on the food supply have included:

1. The market basket survey of pesticide residues in foods sold in Connecticut is conducted in cooperation with the Connecticut Department of Consumer Protection. This has been discussed by Dr. Walter Krol in detail in the preceding article.
2. Investigations of potential product tampering cases, again conducted in cooperation with the Connecticut Department of Consumer Protection.
3. Analyses of samples involved in potential poisoning and/or product tampering cases brought to our attention by local health and police departments.

The quality of the responses from the Department of Analytical Chemistry to these and other topics over the years has highlighted our work well beyond the borders of Connecticut. As a consequence, we are partnering with state and federal agencies to apply our analytical expertise

to a wider variety of topics than before. The one topic of considerable importance in the new initiatives in our Department is that of counter terrorism.

### The National Scene

Leveraging the capacity of federal laboratories with that of state laboratories was acknowledged in the late 1990s through the formation of the Laboratory Response Network (LRN). Under the guidance of the Centers for Disease Control and Prevention (CDC), state departments of public health were funded so as to increase their staffing and instrumental capacities. Initially biological contaminants in clinical samples were targeted by the LRN; chemical and radiological contaminants were added subsequently. Not only were state public health laboratories funded, but hospital laboratories, which handle clinical samples, were also included.

After the events of September 11, 2001, the need to network laboratories whose work involves matrices other than blood, urine, and other tissues of human origin for adulterated substances was recognized. On January 30, 2004, a presidential directive in defense of U.S. Agriculture and Food was issued with the following goals:

- Develop nationwide laboratory networks for food, animal, plant, and water quality that integrate existing Federal and State laboratory resources and utilize standardized protocols and procedures.
- Develop coordinated surveillance and monitoring systems, including international information, for animal disease, plant disease, wildlife disease, food, public health, and water quality to provide early detection of disease, pest, or poisonous agents.

This directive had been anticipated in discussions among scientists from the USDA and FDA. The Food Emergency Response Network (FERN) was developed throughout 2003 and 2004 in meetings of the federal participants and four invited state participants: Florida, Georgia, New York, and Connecticut. During this time, I attended meetings in Athens, Georgia and Rockville, Maryland and participated in numerous conference calls all intended to develop the operating principals of the FERN as enumerated in its Mission Statement: network the nation's food-testing laboratories to enhance the detection and identification of threat agents in food.

Three classes of threat agents were identified—chemical, biological, and radiological. The call went out to state laboratories, which conduct the analysis of food matrices to apply for membership in the FERN under one or more of the threat agent classes. The Department of Analytical Chemistry was accepted into the FERN as a chemistry participant in April 2004.

In the spring of 2005, FERN state chemistry laboratories were invited to submit an application for cooperative agreement funding from the FDA. Eight state laboratories were successful in their funding applications and represent the states of Arizona, California, Florida, Iowa, Minnesota, New Hampshire, Virginia, and the State of Connecticut. We are pleased that the quality of our work has been acknowledged through this agreement and honored that we will contribute to homeland defense with our analytical expertise.

### Analytical Chemistry's Participation in FERN

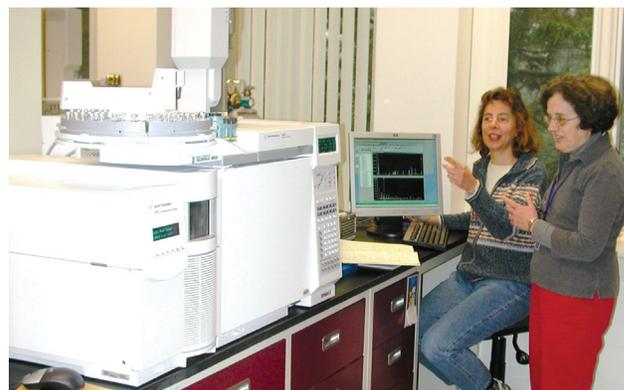
The activities of FERN members are directed toward one of four target areas:

- *Preparedness*: strengthen laboratory capability /capacity
- *Prevention*: enhance federal/state food surveillance programs
- *Response*: assure surge capacity under emergency conditions
- *Recovery*: document post-event food safety to the consumer

Our participation in each of these areas as a FERN CAP laboratory will be presented.

#### *Preparedness*

As part of the CAP funding which we received, our laboratory took delivery of, installed, and now operates several new pieces of equipment. In Figure 1, our new GC/MS (gas chromatograph/mass spectrometer) is shown. Terri Arsenault (left) is the principal operator and she is discussing some results with Dr. MaryJane Incorvia Mattina (right). We have been operating similar equipment for several years as part of our market basket program. The FERN work assigned to our laboratory has priority on this newly acquired GC/MS system.



**Figure 1.**

Figure 2 shows the ICP/MS (inductively coupled plasma/mass spectrometer), which we received as part of the FERN cooperative agreement. Dr. David Stilwell (left) and Craig Musante (right) have learned the operation of this technology, which is new to our laboratory. It allows us to achieve very low detection levels previously not accessible in our facility, as well as to detect elements for which we had no methods. Mamie Pyles works in the background on nitrogen content in feed and fertilizer samples.

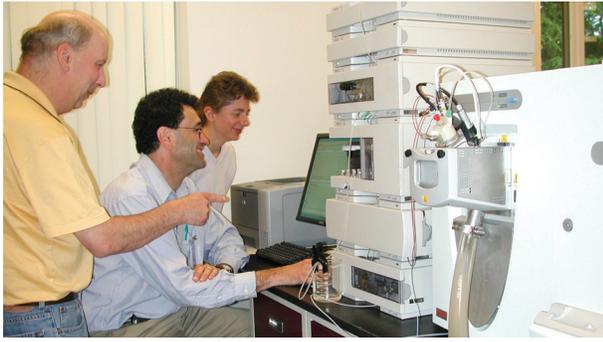


**Figure 2.**

Another new technology acquired through FERN CAP funding, the ELISA system, is shown in Figure 3. One of our new employees, Dr. Christina Robb, has primary responsibility for operation of this equipment. Dr. Robb has joined our staff as a result of our successful CAP application.



**Figure 3.**



**Figure 4.**

In figure 4, we show our LC/MS (liquid chromatography/mass spectrometry) system. This system was purchased in part through funds received from the Connecticut Department of Public Health as part of their counter terrorism efforts. Dr. Brian Eitzer (center) has primary responsibility for the operation of this equipment and he is shown discussing some data with Dr. Walter Krol (left) and Terri Arsenault (right). Since this instrumentation has the capacity for MS<sup>n</sup> techniques, our analytical toolbox has been expanded considerably, not only for food matrices, but also for a wide variety of environmental and miscellaneous samples.

To complete this presentation of newly acquired instrumentation in Figure 5, we show a new GC/MS system. William Berger (left) and Dr. MaryJane Incorvia Mattina (right) stand next to this equipment which permits MS<sup>n</sup> analyses, the same technique as in our LC system shown above.



**Figure 5.**

As an example of analyses on behalf of state agencies which we are now capable of performing resulting from this new equipment and which interface very nicely with our FERN work, I will mention one recent example. A state inspector brought us an open bottle of water from a consumer which contained a partially dissolved capsule and some blue granular material in the water. We were also supplied with a sealed bottle of the same type of water and two intact capsules from the consumer's home. Within a few hours Brian Eitzer was able to demonstrate conclusively that the opened bottle contained the active drug ingredient from one of the two comparison capsules originating with the consumer. Other features from the analysis of the opened sample were consistent with this finding. Such conclusive and rapid response would not have been possible a year ago.

Another example of the interfacing of our state work with our commitments to the FERN program comes from the implementation in our laboratory of new analytical methods supplied to us by our federal partners. Using these methods and our new instrumentation, we are now analyzing for toxic

substances in all appropriate state samples. In addition, Dr. Walter Krol has updated and expanded our list of detectable agrochemicals. The combined outcome from both of these programs, FERN and market basket, is that our list of detectable organic analytes is much larger than it was a short time ago.

The answers which our Department supplies to submitting agencies are only as good as the underlying data. To assure that the data are reliable, our quality assurance includes completion of proficiency tests. Working with our federal partners, we have completed several such tests and will continue to do so in the future.

#### *Prevention*

Surveillance programs can be a strong deterrent to intentional adulteration of the food supply. The Connecticut market basket survey is one such program. Surveillance is also a part of the federal system and Analytical Chemistry has participated in all chemistry-related FERN exercises. In the fall of 2004, our laboratory was one of a select number of state laboratories included in a successful, nation-wide, "proof-of-concept" surveillance exercise. As part of the CAP laboratory group, we are expected to participate in all chemistry surveillance exercises originating from FDA and have done so in 2005 and 2006. In these federal surveillance programs, food samples are collected throughout the US by FDA inspectors and sent to us. Once received in our laboratory, the sample is prepared and analyzed using FDA methods; data are uploaded into a FERN-specific, electronic database.

#### *Response*

Rapid response to an identified criminal event directed toward the food supply is essential to its comprehensive identification. As a FERN CAP laboratory, we have been supplied with instrumentation and additional staff in the anticipation of our full participation in the response phase. Dr. Robb has been introduced above. Another new employee in our Department is John Ranciato, shown in Figure 6, whose employment has been made possible through funding received from the Connecticut Department of Public Health. With this added staff, it will be possible for our department to operate for a limited duration on a 24/7 basis during the response phase.



**Figure 6.**

#### *Recovery*

Following the identification and response phases to an event, the public must be assured of the safety of the food supply. FERN laboratories both within and outside of the CAP will contribute to this phase. We anticipate participating in this phase with our standardized methods and instrumentation.

## The Connecticut Scene

In addition to our increased activities on the national scene resulting from participation in chemical counter terrorism issues, these same issues have resulted in greatly expanded activities within the State of Connecticut between our laboratory and other state agencies. We have made frequent mention of funding received from the Connecticut Department of Public Health, which has resulted in our acquiring new instrumentation and additional staff. We have initiated visits between the two laboratories by DPH scientists and our Analytical Chemists and anticipate some degree of staff cross-training as well.

As a direct result of increased cooperation between our department and DPH, we have learned about the 14th Connecticut Civil Support Team of the Army National Guard. Our interaction with this group of professionals has included visits to each other's facilities and discussions of communication and collaboration. We were privileged to have the 14th CST mobile laboratory available for tours during our April Open House in New Haven, visible in the foreground in Figure 7. In the background of this picture is seen the mobile laboratory from Connecticut Department of Environmental Protection, another state agency with whom we have expanded collaborations.



Figure 7.

## The Future

One of the future challenges in Analytical Chemistry will be to satisfy both our state and federal commitments. In this regard, the overlay of our newly acquired methods and instrumentation will be central. Rather than directing our work along two diverging pathways, our state and federal stakeholders will be best served by the integration of these two sets of activities. In recent months, I have encouraged this approach throughout our service obligations and can report success in this endeavor. Federal and state obligations in the future can and will mutually enhance each other rather than detract from each other.

Dr. MaryJane Incorvia Mattina was born in The Bronx, New York. She graduated in 1966 magna cum laude with a B.A. degree in chemistry from Barnard College, where she was elected to Phi Beta Kappa. She received a Woodrow Wilson fellowship for her graduate work in chemistry at Yale. She received an M.Phil. and her Ph.D. from Yale in 1970. She has taught chemistry at Albertus Magnus College, Simon's Rock College, and Berkshire Community College. She and her husband, Charles, also a chemist, raised three children in western Massachusetts. She returned to New Haven in 1988 to take a position as an Assistant Agricultural Scientist in the Department of Analytical Chemistry at CAES. She was promoted to Associate Scientist in 1990 and was named the head of the Department of Analytical Chemistry in 1992. She has authored or co-authored more than fifty peer-reviewed publications and invited book chapters.

MaryJane's research interests include applications of mass spectrometry to solve questions associated with environmental issues. For the past several years, she has focused on tracking legacy pesticides from their source in soil into and through terrestrial plants with the goal of elucidating the mechanisms responsible for plant accumulation of these chemicals. This work has progressed from field studies into the greenhouse and most recently to smaller scale rhizotron experiments and grafting based experiments. In the near future it is hoped that the genetic foundation for the observations can be determined.



The mission of The Connecticut Agricultural Experiment Station is to develop, advance, and disseminate scientific knowledge, improve agricultural productivity and environmental quality, protect plants, and enhance human health and well-being through research for the benefit of Connecticut residents and the nation. Seeking solutions across a variety of disciplines for the benefit of urban, suburban, and rural communities, Station scientists remain committed to “Putting Science to Work for Society”, a motto as relevant today as it was at our founding in 1875. [www.ct.gov/caes](http://www.ct.gov/caes)

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