How Lloyd G. Keirstead uses gas chromatography to find residues ... see page 2

Residue Determination By Gas Chromatography 2
Our Crops Are Like Canaries 3
Can We Control Succession In Field and Forest? 4
Bacillus thuringiensis 6
Efficient Agriculture and Cities 8

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN
RESIDUE DETERMINATION BY GAS CHROMATOGRAPHY

Lloyd G. Keirstead

The measurement of pesticide residues involves three basic steps — extraction, clean up, and measurement. The first two steps are extremely important, but perhaps we may content ourselves to look at some of the things which have happened to the measurement procedure.

When DDT came into use, the customary measurement procedure was to dissolve the residue in a suitable solvent, cleave off the chlorine and measure it. DDT is almost exactly half chlorine by weight and, therefore, it was only necessary to multiply the chlorine figure by two.

This happy situation did not last long. BHC, chlordane, dieldrin, endrin, kelp thane, methoxychlor, and other chlorinated pesticides also lost their chlorine with appropriate treatment. It was impossible to know what compound was actually present unless the previous history of the sample was known.

This led to the color era. An ingenious chemist can find a chemical, or chemicals, which will react with a pesticide to give a colored compound. This colored material can be dissolved and the color measured. Hardly a pesticide exists for which a color method has not been published.

Many Tests May Be Necessary

The color methods are often quite specific and will measure small amounts. The disadvantage comes in the analysis of material containing a mixture when the previous history is not known. Conducting a long series of color reactions takes a lot of time, and many of the tests prove unnecessary. The tests can also be tedious and often require both special reagents and special equipment.

The ideal is a procedure which will tell what is there and how much. Paper chromatography comes close. This technique is based on the finding that pesticides can be made to migrate up a sheet of filter paper at different rates. The paper is first impregnated with a suitable material such as mineral oil. The pesticides in solution are transferred to the paper in dots about an inch from the end, and the solvent allowed to evaporate. The end below the spots is dipped in a solvent; this part of the operation is run in a closed tank.

The competition between the solvent climbing the paper wick and the material impregnated causes the pesticides to migrate at different speeds. When the solvent has gone to the top, the paper is removed and sprayed with reagents which make the spots visible.

Standards are run on the same paper. If a spot climbs to the same height as DDT, it is assumed that it is DDT. Amounts can be estimated by putting on standards of different amounts and comparing the size and intensity of the spots with those produced by the sample. This is an elegant identification method, but relies too much on human judgment in the quantitative estimation.

Gas liquid chromatography does the same job, is much more sensitive, and the quantitative measurement is reduced to simple manipulations which lessen the human error. The drug consists of a tube which has been filled with an inert material that has been impregnated, usually with a silicone grease or a silicone rubber.

The filled tube is placed in an oven which is heated in the neighborhood of 200°C, and one end of the tube is connected to a cylinder of nitrogen which is allowed to flow through at the desired rate. A small measured solution of pesticides is injected. The heat vaporizes the pesticides which are wafted through by the nitrogen. Competition between the nitrogen and the impregnated packing causes the compounds to emerge at different times.

Recorded Electronically

The remaining requirement is a detector to "see" the chlorinated pesticides and some of the other types. These detectors contain a small amount of a radioactive element, often tritium, which produces a charge so that a current flows when a voltage is applied between the anode and the cathode. This current remains steady until a suitable material comes along which acts as a "blotter" for the charged particles and causes the current to fall off. Chlorinated pesticides are very effective "blotters." The electronic experts have devised the necessary equipment to have this "blotting" appear as blips on a recording chart which is traveling at a constant rate. The time of emergence indicates what the substance is, and the area under the blip, when compared to suitable standards, allows one to measure the amount.

We bought a gas chromatograph last summer to supplement the other techniques we use. It is proving to be one of

Frontiers of Plant Science
Our Crops Are Like Canaries

Saul Rich

TRADITIONALLY, canaries are used in mines to warn of poisonous fumes. By watching the canaries, miners can tell when the air in the mines may be polluted. Similarly, our crops have been warning us that our air is polluted. In recent years, the foliage of many plants has been injured by an air pollutant called ozone.

Ozone can be considered abnormal oxygen. The molecular oxygen we breathe is made of two oxygen atoms (O₂), but ozone has three oxygen atoms (O₃). This extra oxygen atom makes ozone highly reactive and germicidal. Ozone has been used in place of chlorine for purifying water.

The leaves of some of our crops, such as tobacco and tomatoes, are so sensitive that they may be injured by as little as 1 part of ozone per 10 million parts of air. This is about 3 tablespoonsful of ozone gas in a houseful of air.

What is the source of our crop-damaging ozone? Scientists in California have found that the principal offender is the automobile. Exhaust fumes contaminate the air with nitrogen dioxide, as well as much unburned gasoline. It is believed that when sunlight strikes the mixture of these two gases, ozone is produced.

Fortunately, ozone is unstable and begins to break down quickly. But ozone accumulates if it is produced faster than it can decompose. On sunny mornings, ozone builds up in the air as automobile traffic becomes heavy. The accumulation of ozone ends only when the sun sets. Since no new ozone is produced after sunset, the ozone already in the air gradually disappears before the following morning.

Although ozone is produced around metropolitan areas on every sunny day, it only becomes a problem on certain days. The sun warms the earth, resulting in warm air next to the ground. The warmed air containing the unwanted ozone ordinarily can rise into the cooler air above, and is replaced by clean air. This system of air cleansing takes place only so long as the upper air is cooler than the air near the ground. However, on our very finest summer days the upper air becomes warm, trapping the air at ground level. When this occurs, fumes and ozone accumulate, reaching concentrations that may damage our crops.

OZONE DAMAGE IS COSTLY

Ozone damage has been reported on at least 57 different kinds of plants, including row crops, vine crops, ornamentals, cereals, and trees. In Connecticut, we have seen such injury on tobacco, tomatoes, beans, spinach, petunias, and many other plants. We estimate that ozone damage costs our growers at least $500,000 to more than $1,000,000 each year.

How can we protect susceptible plants from ozone? One way this may be done is to remove or destroy the excess ozone before it reaches the plant. For the past few years we have been attempting to do this by treating plants with chemical compounds called antiozonants. We have also used these compounds as treatments for cloth covers, such as the tobacco shade tents. These antiozonants destroy ozone, but unfortunately are used up themselves in the protective process. We are continuing with our efforts to find better antiozonants and better ways to use them.

We also know something else about ozone damage to plants that may help us to protect our crops. We know that susceptible plants are injured by ozone only when their stomata, or breathing pores, are open. Ozone must pass through these stomata in order to reach the tender interior of the leaves. The stomata are very tiny and very numerous. A square inch of tobacco leaf may be perforated with 50,000 stomata on its upper surface and 100,000 on its lower surface. Each stomatal pore is lipped by two guard cells that swell and shrink to control the size of the opening.

If we can close the stomata before, or early in a high-ozone period, we should be able to protect the plants. This approach to the problem became possible when Dr. Israel Zelitch in our Biochemistry Department discovered that some compounds can cause open stomata to close, and prevent the opening of already closed stomata. Open stomata close within 30 to 60 minutes after being treated, and may stay closed for 3 to 7 days.

With these compounds, we have been able to protect susceptible plants in the laboratory and greenhouse from artificial irritation with ozone, and during natural high-ozone periods. Protecting plants from ozone by chemical regulation of stomata looks sufficiently promising to warrant field trials during the coming season.

As time goes on, our growing population will drive more and more automobiles, and it is obvious that air pollution damage to crops will become increasingly serious. We plan to explore every means for protecting our plants from this damage.
Can We Control Succession
In Field and Forest?

Before colonial times, Connecticut was a forest. With the decrease in farm acreage in New England, another forest has come to occupy much of the State.

Reestablishment of forest on the abandoned fields takes place in a series of stages, each with characteristic plants and animals. The earliest stage consists of grassland with isolated pioneer trees such as sumac, juniper, red cedar, or gray birch. At a later stage we find that these trees, along with the grasses and wild flowers, have disappeared and have been replaced by a wide variety of trees including oaks, maples, and tulip-poplar, with perhaps an understory of small trees and shrubs. Later still, we see the succession reach the climax or terminal phase, dense forest, where all but oak and hemlock tend to be slowly eliminated.

Forest succession, particularly in the later stages, is a slow process. Nevertheless, fields representing each step in re-colonization can be seen today throughout the State. Succession appears to be moving towards the same conclusion over a large area.

Within this changing scene, Suburban Forest Man has chosen to make his home. Some woodland residents will eagerly anticipate the approach of dense forest and seclusion. Others may want a varied rural landscape of both field and forest to remain. For a number of reasons, they may wish to discourage succession from going "all the way" in suburbia.

As the forest grows older, fewer tree species are evident. This loss of diversity has several disadvantages. The species eliminated include the attractive flowering trees, shrubs, and herbaceous plants. The preponderance of oak produces a canopy susceptible to caterpillar infestation. There is an additional aesthetic objection to widespread forest. Much of Connecticut is flat or gently rolling, and the landscape is easily obscured by forest. In some areas, vistas are confined to a few old pastures or along a highway. Over large areas of the State, openings in the forest are scarce and they are dwindling rapidly.

To maintain road margins, non-selective eradication of invading trees may be necessary. However, it is equally desirable that we have also at our command, more subtle techniques for arresting succession or diverting its course towards new ends.

Control of succession is as old as agriculture. Man has used a variety of means — fire, the axe, the scythe, the plough, the grazing animal—to preserve the early phases of succession. These methods involve an expenditure in time, energy, and capital which we rarely consider justified today. As an alternative, over the last decade, we have come to rely

To compare the ability of seedlings to penetrate a barrier of shade, Dr. Grime grew them in plastic cylinders painted to give gradients of sunlight. Green overprint shows height of the painted area in centimeters. The weight in grams of storage material in a single seed is shown below each tree name. (D indicates that the seedlings died soon after planting.)

---

Some of those who live in the Suburban Forest eagerly anticipate the approach of dense forest and seclusion. Others may want a varied rural landscape of both field and forest to remain.
Japanese chestnut seedlings grown in clear and partly painted plastic cylinders, with a no-treatment check at the left. Results with five of the ten species Dr. Grime studied are shown in the chart below.

upon chemical treatments for control measures. Silvicides have proved to be efficient for the general elimination of tree seedlings from grassland, and compounds which are under development give promise of techniques selective between conifers and deciduous species.

Compounds now available have the disadvantage of causing damage to other broadleafed species when applied generally. Where the number to be eliminated is small, this difficulty may be overcome by applying silvicide to individual seedlings.

As the Suburban Forest growth is consolidated, this method of control becomes less practicable. With the rising number of seed-bearing trees, more and more areas become saturated annually with seed of birch and maple. The growing invasion is not confined to wind-blown seeds; birds perennially bring new seed of other trees such as black cherry, red cedar, and oak. In these circumstances the use of silvicides may become tedious. Even so, for the owner of a small acreage, frequent and judicious use of herbicide and rotary mower may continue to be the best method available. For larger areas, and in particular for highway margins, it would be of great advantage if we could stabilize grassland or low shrub communities by methods which remain effective over long periods.

When chemical methods have disadvantages, it is customary to call forth the ecologist and get out the well-worn Aladdin's Lamp, "Biological Control." In this instance, however, skepticism is out of place, for an ecological approach does give promise of alternative methods.

In current experiments at the Station Farm we are evaluating several methods of biological control by testing their effectiveness on tree seedlings sown into an old field.

Ecological studies indicate that invasion of grassland and shrubbery by many of the pioneer trees is limited to small patches of bared soil which occur in gaps in the vegetation following erosion or disturbance by animals in the soil or by man. In a recent experiment with seedlings of ten different trees we were able to show that several species which appear early in succession cannot penetrate through shade of the type afforded by dense grassland.

It seems possible, therefore, to resist invasion of these species by ensuring that few openings occur in the vegetation and that when gaps do occur they are rapidly healed. Sometimes this can be achieved simply by application of fertilizer to encourage vigorous growth in the grassland plants. Our experiments show, however, that we cannot expect this method to be effective against large seeded plants such as red oak and Japanese chestnut.

In another method under examination at New Haven, we are attempting to confine the roots of tree seedlings to the surface layer of the soil by means of a steel mesh buried below the turf. Preliminary experiments suggest that it is possible to strangle tree roots without affecting root penetration by grasses. By this means we may be able to prevent tree establishment by exposing the invading seedlings to drought and severe competition from the grass roots which are concentrated close to the soil surface. If successful, this method will provide a method of perpetuating grassland and may eventually find some application in lawn management.
Bacillus thuringiensis
Problems and Prospects

In the annals of plant protection, the chapters on non-chemical insecticides warrant careful study. One of these chapters — still clearly incomplete — deals with Bacillus thuringiensis, a bacterium long known for its ability to kill certain insects, particularly lepidopterous larvae.

For several years we have been interested in this bacterium as a possible control for some of our defoliating caterpillars. Low toxicity to wildlife makes it particularly useful against pests of woodlands and shade trees where problems with insecticides may arise. People living in wooded areas are well aware of the damage done by heavy infestations of defoliating caterpillars feeding uncontrolled.

In 1961 Dr. Richard J. Quinton and I tested wettable powder sprays of B. thuringiensis on apple trees heavily infested with the fall cankerworm. The high doses we used gave satisfactory protection, while untreated trees were completely defoliated.

In 1963 Dr. Stephen W. Hitchcock and I tested an experimental liquid suspension of B. thuringiensis against cankerworms and the gypsy moth. The sprays were applied by helicopter to 50-acre plots in a mixed hardwood forest. The results of this experiment were somewhat erratic and suggest that further experimental work is needed before large-scale control with this bacterium is feasible. Station Bulletin 665, available on request, tells the story of the 1963 experiments.

The history of B. thuringiensis shows that Berliner first isolated the bacterium from diseased larvae of the Mediterranean flour moth about 50 years ago. In the years that followed his publication periodic attempts to use this organism for insect control met little success. Thus in 1926 to 1930 B. thuringiensis proved effective in the laboratory but not in the field in tests on corn borer and gypsy moth. The successful use of B. thuringiensis on alfalfa caterpillars by Steinhaus of California revived interest in its possibilities.

The most important advance, however, was a rediscovery by Hannay in 1953 of the crystal which Berliner had described in 1915.

Originally it was supposed that after ingestion the spores of this bacterium multiply rapidly in the gut and eventually break through into the body cavity, causing a fatal septicemia. We now know that the sequence of events is much more complicated.

Hannay found that as each vegetative cell sporulated, a resistant spore is formed at one end of the cell, and a diamond-shaped crystal is formed at the other. These two bodies are then released by the disruption of the old cell wall. The rediscovery of the crystal, and the more important observation that these crystals might be connected with the pathogenicity of Bacillus thuringiensis, brought rapid advances in research. Since 1953 it has been found that B. thuringiensis produces at least four other toxins as well as the toxic crystal. Most attention has been given the toxic crystal.

Heimpel and Angus have grouped the lepidopterous larvae into three types according to their response to the crystal toxin. In the first type, the gut of the larva becomes paralyzed 20 to 30 minutes after ingestion of the toxin, followed by total paralysis in 1 to 7 hours. The silkworm and the tomato hornworm are representatives of this type.

The greatest number of species belong to the second type. Only the gut becomes paralyzed; the insect ceases feeding, wanders about, and usually dies in 2 to 4 days. The spores ingested with the crystals germinate, and toxic metabolites produced during this vegetative stage assist in killing the insect.

Finally, the response of the third type of larvae to B. thuringiensis is the most complicated. Investigation of this type led to the discovery of a soluble toxic material present in the growth medium. The combination of this toxin together with the spores and crystals is necessary to produce high mortality to the Mediterranean flour moth. This soluble toxin has been commonly lost during preparation of the spore crystal formulation. The spores or toxic crystals used alone cause only slight mortality in this third type of larvae.

The crystal toxin is alkali-soluble. To be effective it must be dissolved in the gut of the larva. Thus only those larvae with a suitably high alkaline gut content are susceptible to the crystal of B. thuringiensis. Resistance in certain species may be

Gypsy moth defoliation in Mettacuck State Forest, Watertown, June 28, 1962.
explained: the gut contents are not alkaline enough to break down the crystal.

*Bacillus thuringiensis* is applied more like an insecticide than are some biological control agents since it does not maintain itself in nature by causing continuous reinfection. In contrast, *Bacillus popilliae*, the milky spore disease organism in larvae of the Japanese beetle, remains effective in the soil for many years. The effective life of *B. thuringiensis* is about 1 week. Repeated applications are therefore commonly necessary.

Since the discovery of the crystals, upwards of 40 separate strains of crystal-forming bacteria have been isolated from various parts of the world. Most of these differ widely in their pathogenicity to insects. At present only one strain of *B. thuringiensis* is being produced commercially for insect control.

Strains may eventually be selected for their virulence to specific insects or groups of insects. This development of new strains is necessarily slow, since each insect must be studied in relation to each strain under consideration.

A drawback in the use of *B. thuringiensis* is the rather large amount of the material needed to kill certain insects. One recent line of investigation is a search for additives that might enhance or increase the effect of the bacterium or, conversely, make the insect more susceptible to the spores and crystals. Dr. Robert C. Wallis and I have found that dilute sprays of boric acid in combination with *B. thuringiensis* approximately doubled the mortality of gypsy moth larvae under laboratory conditions. Boric acid used alone caused no mortality. The practical value of this observation, if any, awaits evaluation in the field.

### New Publications

The publications listed below have been issued by the Station since you last received *Frontiers*. Address requests for copies to Publications, The Connecticut Agricultural Experiment Station, Box 1106, New Haven, Connecticut 06504.

**Climatology**

  - Christopher Bingham.

**Entomology**

- C 224 *Insects in Houses.* J. Peter Johnson and Neely Turner.
- C 225 *Dogwood Club Gall.* John C. Schread.
- B 665 *Field Tests with an Aerial Application of Bacillus thuringiensis.* Charles C. Doane and Stephen W. Hitchcock.

**Plant Pathology**

- B 663 *Perspectives of Biochemical Plant Pathology.* (Technical papers presented at the 75th anniversary of the Department of Plant Pathology and Botany, New Haven, May 1963.)
  - Edited by Saul Rich.

**Reports on Inspections**


**Soils**


### IN THE NEWS

**Peter R. Day**

Dr. Peter Rodney Day has been appointed to head the Department of Genetics by the Station Board of Control. He will begin work here on July 1.

Presently an associate professor at Ohio State University, Dr. Day was graduated from Birkbeck College, University of London, in 1950. After 4 years at the John Innes Institute in Bayfordbury, he received the Ph. D. degree from the University of London.

A Commonwealth Fund Fellow at the University of Wisconsin from 1954 to 1956, he returned to John Innes in 1957 as Senior Scientific Officer in the Genetics Department, a position he held until 1963.

**John F. Anderson**

The biology of mosquitoes is the principal field of inquiry for Dr. John F. Anderson, new member of the Station staff in the Department of Entomology. Last year Dr. Anderson (Ph. D. Illinois, 1963) was a Louisiana State University Fellow in tropical medicine and parasitology.

His previous work on effects of temperature on mosquitoes has revealed unexpected changes in sex. Continuation of this and related research may lead to new knowledge of mosquitoes.

**James G. Horsfall**

Dr. James G. Horsfall served as chairman of a committee appointed by President James A. Perkins of Cornell University to lay the groundwork for a study of the future role of the New York State College of Agriculture.
From the Director

James E. Herpfel

Our name is The Connecticut Agricultural Experiment Station. Some thoughtful citizens have told us that the word Agricultural in our name suggests that perhaps we operate only a gravy train for a few farmers. They think that we should change our name to some variant of Biological Science Institute because we are biological scientists.

Why have we not taken their advice? Numerous colleges of agriculture have changed their names.

Our answer is that Agricultural is still properly one of our middle names. True, farmers may become fewer, but the importance of agriculture increases with every passing day as this nation adds 7,000 more mouths to feed.

Then, too, Agricultural Experiment Stations are charged with improving agriculture. Biological Science Institutes are not. And this urban nation depends mightily upon efficient agriculture. The gravy train of research useful in agriculture is for all the citizens who eat.

We need not dwell on the population explosion nor on the clear responsibility of an Experiment Station to contribute toward more efficient agriculture. The benefits of efficient agriculture to the millions of urbanized Americans may not be so obvious.

But I think that Connecticut people understand. Everybody knows that this is an urban state. More than a century ago, when the trickle across the Appalachians became a flood, it became clear that Connecticut must urbanize or die, and urbanize it did.

A citizen of an agrarian society produces his own food. A citizen of an urban society depends upon someone else for his food. On that account a society can urbanize itself only as fast as it can increase its agricultural efficiency.

Some thoughtful citizens of Connecticut must have sensed that basic element in modern society nearly a century ago, for in 1875 this state established the first Agricultural Experiment Station in the nation and instructed it to put science to work for agriculture. The bill was steered through the legislature by a suburbanite lawyer of New Haven, James J. Webb.

Invention for Making Inventions

Thus (for $2000) Connecticut invented a kind of institution for making inventions solely needed by a state and nation in the process of urbanization. Other states quickly followed the lead of Connecticut, and within 15 years the principle of state and federal support of agricultural research was a policy of government, as it is today in every state and in every nation.

Experiment Stations create inventions for producing food efficiently, just as industry creates inventions for producing goods efficiently. Henry Ford, an inventor, perfected mass production of machines. He and his contemporaries made modern urban areas inevitable. The late Donald Jones of this Station was also an inventor. He perfected a technique for mass production of food. He and his contemporaries made our modern urban complexes possible.

"In the sweat of thy face shalt thou eat bread," says the Bible. Because agriculture in America is efficient, 27 people now eat by the sweat of one farmer's brow. The other 26 may sweat when the air-conditioning fails, when they play tennis, or when they work in a steel mill. But they are not needed as field hands.

No one seriously suggests that we smash up machines so that more people can do more work with bulb strength and ignorance. But a recent writer of a Letter to the Editor came close. He suggested that use of synthetic agricultural chemicals be abandoned, citing this gain: creation of thousands and thousands of new jobs.

Premier Khruchochetz set forth a different view last February in a policy speech on agriculture. He said: "The chief thing for us today is the production of mineral fertilizers, herbicides, various additives for animal husbandry, growth stimulators, and others. Chemistry has a very rich assortment of products that must be put at the service of agriculture."

So says Mr. Khruchochetz, who some years ago took the advice of an Iowa farmer, Roswell Garth, and really put hybrid corn to work on Soviet farms. Mr. Khruchochetz is well aware that one American farmer feeds 27 of his countrymen, and he has learned some of the reasons that explain how this is possible.

We may not think that he understands the reasons why.

You may have seen the cartoon that points one of the headaches that be-devil an urbanizing Russia. One frustrated commissar says to another: "Damn those Yankees. They told us they were making rockets, but they were growing corn all the time."

We'll keep Agricultural in our name.