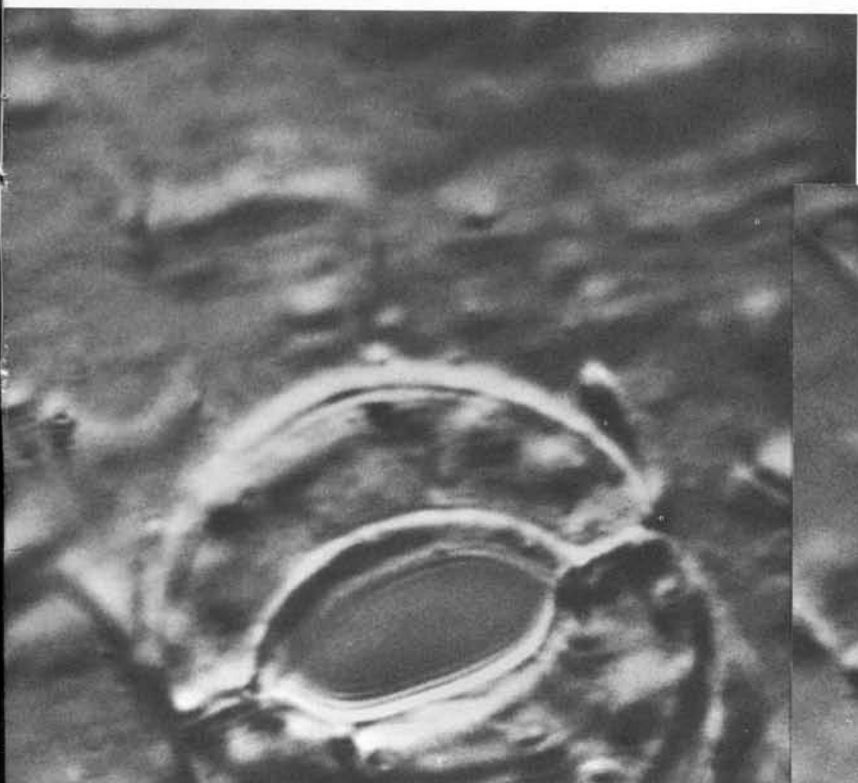
SPRING 1968

Frontiers of Plant Science

20th Anniversary Issue



Open (above) and partly closed stomates in tobacco leaf. For stories on life processes in leaves see pages 4 and 8.

Photos by Fritz Goro, LIFE Magazine

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION NEW HAVEN

Established in 1875 by the General Assembly

F RONTIERS OF PLANT SCIENCE came onto the Connecticut scene 20 years ago. In the new journal, the new Director of the Station promised that we would "eliminate all ponderous detail and try to present our story in such a way that its meaning will be readily apparent." That is still our intent.

We have tried to show how we use your investment in plant science research to help keep Connecticut clean, green, and productive.

As is obvious to the elders, Connecticut has changed mightily since 1948 when publication of FRONTIERS began. Our population has increased by about 1 million in 20 years, compared with 270 years to reach the 1 million mark and 45 more years to reach the 2 million mark.

We live in the fourth most densely populated State, described by an editorial writer as "a pleasant island that is doomed to be inundated by the rising population of the eastern United States. . . . A few more years, and oldtimers will say to their grandchildren, 'I can remember when there was a wild tree only a mile from my house.'"*

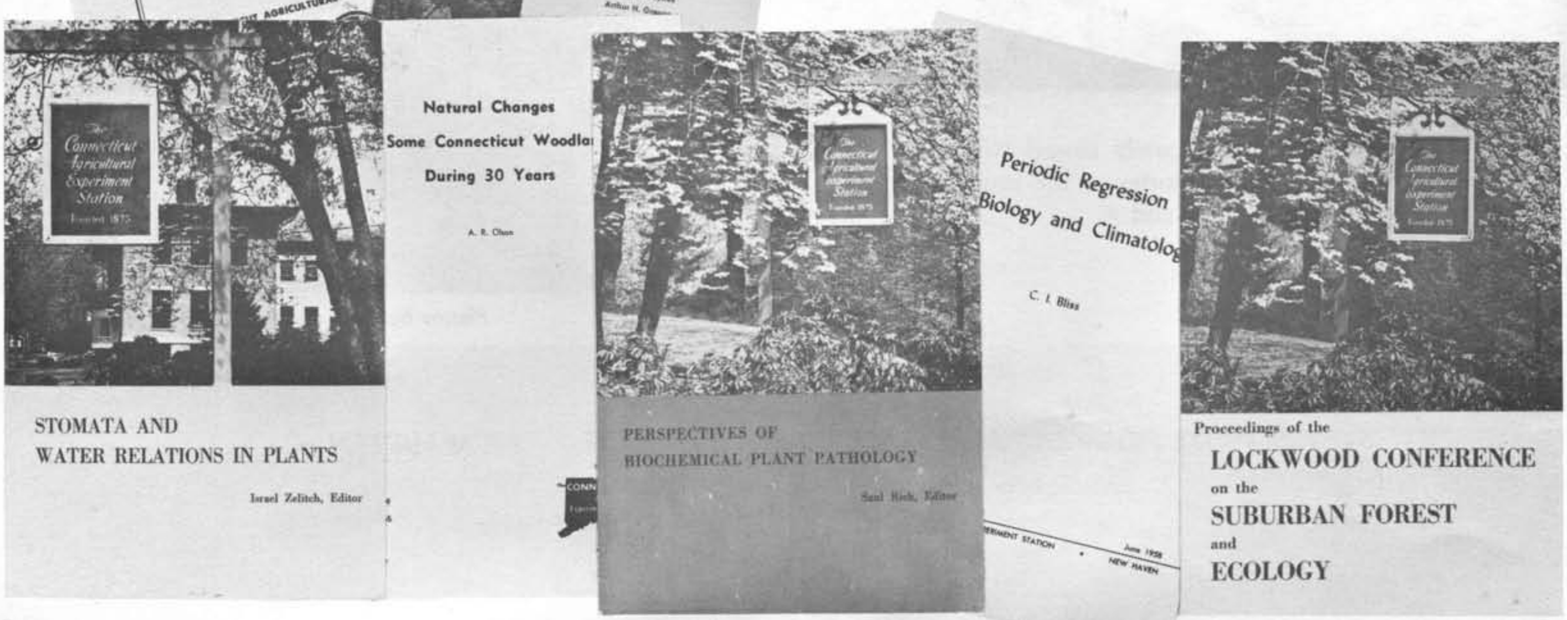
We at the Station hope that things never come to such a dismal pass. Perhaps our addiction to understanding how and why plants grow and fruit and return to the eternal mold accounts for our faith that Connecticut will not become a sea of concrete and blacktop—an airport and parking lot for Megalopolis East.

Meanwhile, the Station continues research, as Director James G. Horsfall has written, "on how to feed all the American animals that cackle and moo and talk."

And to do what we can to make sure that it is not a mile from any Connecticut citizen's house to a wild tree.

The cover pages of typical Station publications among 300 issued during the past 20 years suggest some of the ways we move our product to those who use it. Not shown are more than 1,500 technical papers published since 1948 in scientific and professional journals to convey ideas from mind to mind.

* *Hartford Times*, April 15, 1968.



In Refrigerated Foods

Quick Test Reveals Contamination By "Cold-Loving" Bacteria

Lester Hankin
Biochemistry

THE HOUSEWIFE who takes pride in her culinary artistry is distressed to find that a refrigerated food has developed an off-flavor or odor. One cause for this deterioration is the presence of bacteria able to grow at refrigeration temperatures. These cold-loving bacteria, known scientifically as psychrophiles, are equally troublesome to the food processor, for they can sharply reduce the "shelf life" of foods.

The usual tests for the presence of these psychrophilic bacteria often take so long that the food may be unpalatable (and discarded) before the data are obtained. We have been able to apply an oxidase test for these bacteria and so reduce from 7 days (or much longer) to 2 days the time required to estimate perishability of milk. The test is adaptable to other food products.

With the 2-day test, samples contaminated with psychrophilic bacteria can be withheld from sale and the source of contamination promptly eliminated.

To understand how the new test works, it is necessary to know something about the bacteria involved. Of all the psychrophiles, the nonpathogenic pseudomonads certainly can lay claim to being the most obnoxious and the most troublesome. Different species of this genus produce off-flavors and odors described as fruity, musty, skunk-like, fishy, and many more just as unpleasant. Other flavor criticisms such as stale, lacks freshness, and unclean may also be due to low levels of pseudomonad contamination. These cold-loving bacteria are becoming more troublesome as cold-storage periods lengthen. This is especially true with dairy products—milk may be collected from the farm every-other-day and home delivery made only two or three times a week.

In the development of analytical tests it is often necessary to draw on knowledge from different disciplines of science. Data from clinical microbiology, biochemistry, bacteriology, and food science were used in adapting the oxidase test to estimate storage life.

The quick test for psychrophiles is based on three propositions. First, pseudomonads account for most of the problems caused by psychrophiles in refrigerated food. Second, the pseudomonads can be specifically detected in a mixed bacterial population. We have shown that these organisms can be selectively enumerated. Third, we assume that a positive correlation exists between the number of pseudomonads present in a sample and the data obtained with the present lengthy test for all psychrophiles; this we have confirmed.

We used milk as the test food since it is in plentiful daily supply and may be readily contaminated with cold-loving bacteria.

Briefly, the rapid test is conducted as follows. Petri dish cultures are made for the total bacterial population of the food sample. This takes two days. Most of this time is required to allow the bacteria to grow into visible colonies. After the colonies on the petri dish are counted, the same dish is flooded with the oxidase test reagent. After 15 minutes the colonies are again counted, enumerating only those which have turned blue (oxidase positive colonies). This blue color is the end product of a biochemical reaction of the bacteria. Pseudomonads have a strong cytochrome *c* oxidase enzyme system, a feature lacking, or weak, in most other bacteria. This enzyme system is able to catalyze the coupling of two organic compounds (the oxidase test reagent) to form the blue color.

The oxidase test thus yields information which requires little additional laboratory time, since fuller use is made of tests regularly conducted in food laboratories. The practical value of the test far outweighs the small amount of time needed for the oxidase test.

The oxidase test has also been used on raw milk from Connecticut dairy farms. Pseudomonads are prevalent in soil and water and can find their way into the milk and equipment used on the farm. Milk with off-flavor is rejected at the dairy plant at a loss to the farmer.

The data provided by this test will enable both control officials and dairy fieldmen to pinpoint sources of possible contamination and suggest sanitation procedures so that the farmer can produce milk of better quality. The ratio between the oxidase positive organisms and the total bacterial count of the sample provides this information.

In some cases we have been able to predict a potential build-up of detrimental bacteria 4 weeks in advance of standard bacterial tests.

Information provided by the oxidase test will help farmers, processors, and the consumer by more rapidly determining the shelf life of the product. The development of methods of this type is another example of research at this Station to give Connecticut consumers the best possible food products.

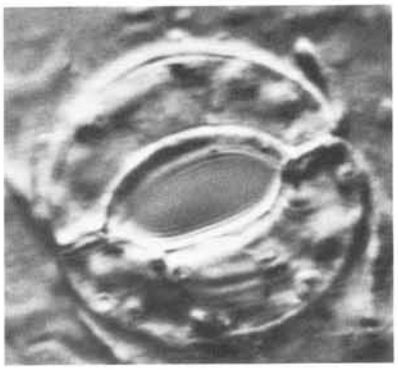


Photo by Fritz Goro, LIFE Magazine

Can We Make Plants With More Efficient Photosynthesis?

P. R. Day
Genetics

and

Israel Zelitch
Biochemistry

ALL LIVING GREEN PLANTS carry out photosynthesis and respiration. In photosynthesis carbon dioxide comes into the plant. In respiration it goes out. Thus, both processes involve exchange of carbon dioxide between the plant and the atmosphere. Since photosynthesis occurs only in the light, it can be measured in the light by metering the ingoing carbon dioxide. Since respiration occurs in the dark, it can be measured in the dark by metering the output of carbon dioxide. The question is, does respiration occur in the light, and if so, how can it be measured? How can one distinguish between the carbon dioxide that comes into the leaf for use in photosynthesis and that which goes out of the leaf from the process of respiration? It is generally assumed that respiration does occur in the daylight. This has been demonstrated in many, but not all, plants in recent years and the process is called photorespiration. Photorespiration—as its name implies—occurs only in the light. This article describes some new work on photorespiration and considers its significance in relation to photosynthesis.

One of the most basic processes for life is photosynthesis. Energy from the sun, trapped and harnessed by the chlorophyll in the leaves and stems of plants, is used to convert carbon dioxide from the air into food materials and at the same time oxygen is released to the atmosphere. These food materials are used for growth of the plant itself including formation of new leaves, roots, and seed and, of course, food for ourselves in the form of the crops and livestock forage we harvest.

The average concentration of carbon dioxide in the atmosphere is about 3 parts in 10,000. Carbon dioxide enters the leaves of a plant through small pores, diffuses into the cells, and moves in solution into the chloroplasts where it begins its transformation into carbohydrates.

Sensitive instruments can tell us how much carbon dioxide is taken up by a known leaf area in a given time. When a leaf is enclosed in a transparent air-tight box and placed in a strong light, carbon dioxide from the air surrounding the leaf is absorbed and the concentration of carbon dioxide inside the box falls. With tobacco and many other plants the concentration falls to about 0.5 parts in 10,000, but no lower. (The instruments can measure one-fiftieth of that level.) At this concentration it seems that carbon dioxide uptake by photosynthesis and carbon dioxide release by respiration are in balance so that the concentration in the enclosed space does not change.

On the other hand, similar experiments with corn show that the carbon dioxide concentration is reduced nearly to zero (less than 0.05 parts in 10,000). Evidently corn leaves release considerably less carbon dioxide in the light. We can also com-

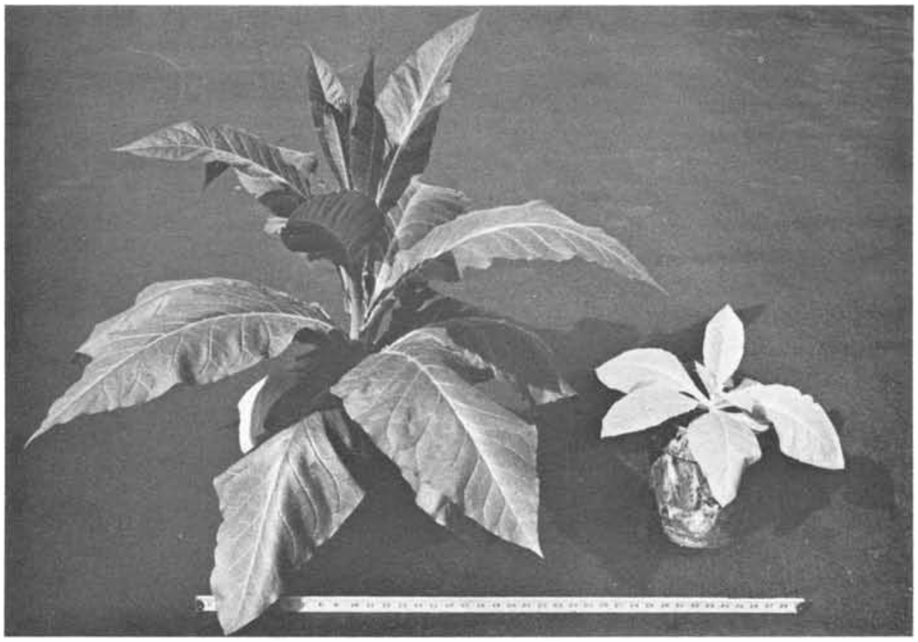
The amount of carbon dioxide taken up by tobacco and corn leaves at 30°C in light of 1,500 f.c. intensity

Plants	*Units* of carbon dioxide*	*Number of determinations*
Green (su su)	16.8±0.4	22
Yellow (Su su)	10.8±0.6	21
Havana Seed	13.6±0.5	17
Corn	26.5±0.7	8

* Milligrams per square decimeter per hour.

pare the relative amounts of carbon dioxide taken up by corn and tobacco leaves from a monitored air stream in the light under normal atmospheric conditions. We find that corn leaves absorb nearly twice as much carbon dioxide per unit area as tobacco leaves.

If a leaf gives off a great deal of carbon dioxide in the light, it is unable to fix as much as a leaf which gives off but little. A very large number of temperate-zone crops, including wheat, barley, rye, soybeans, tomato and potato, give off significant amounts of carbon dioxide in the light. The question arises, can such crops be manipulated so that they give off less carbon dioxide in the light and are therefore more efficient per unit of leaf area? If so, then inherent limits to yield might be raised.

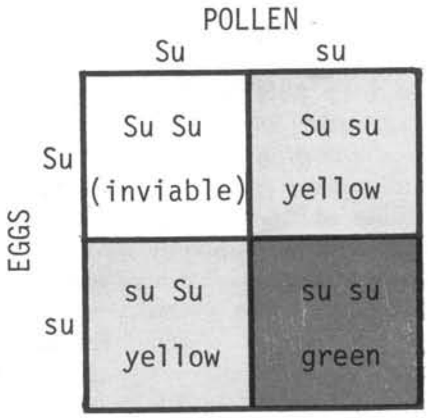


Tobacco plants 14 weeks old, left—dark green *su su;* right—yellow *Su su.*

To answer this question we have to determine why corn does not respire very much in the light although tobacco does. The evolution of carbon dioxide in the light is the result of photorespiration. This process is not the same as the respiration which occurs in darkness. Photorespiration in fact produces several times as much carbon dioxide as dark respiration, and it is generated in an entirely different way. For example a tobacco leaf in light, flushed with a rapid stream of carbon dioxide free air, loses more carbon dioxide than a similar leaf in darkness.

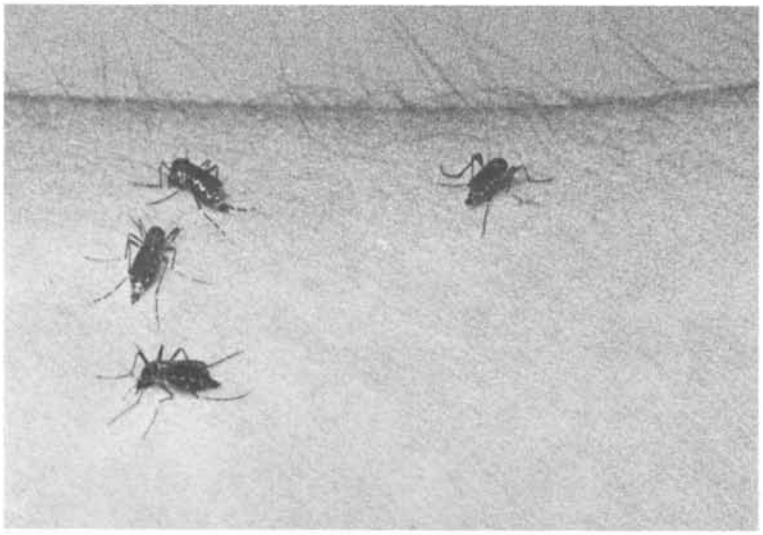
Some experiments with leaf discs strongly suggest that the carbon dioxide released during photorespiration is derived from glycolic acid, an early intermediate product of photosynthesis. This substance is broken down by an enzyme, which is present in sufficient amounts to explain the rates observed, with the ultimate release of carbon dioxide inside the leaf. In leaf disc experiments, chemical inhibitors which poison this enzyme can be shown to suppress photorespiration entirely in tobacco, making it like the corn plant in this respect. Unfortunately the inhibitors have deleterious side effects so that they cannot be sprayed over growing crops. The action of the inhibitors, however, suggests that the desirable effect might be obtained by genetic means. Accordingly we began a search for tobacco plants with below-normal photorespiration rates, hence more efficient in their use of carbon dioxide.

A description of a yellow tobacco mutant (G. H. Schmid & H. Gaffron, J. Gen. Physiol. *50,* 563, 1967) suggested that it might be the type of plant we were looking for. This mutant, a dominant, is only viable when heterozygous, that is hybrid with respect to one pair of genes. Every time seed from such a plant is sown some of the seedlings are normal dark green. When a yellow plant (Su su) is self-pollinated three kinds of fertilized eggs arise:



Each fertilized egg develops into an embryo plant inside a seed. When the seeds are sown one-fourth (Su Su) of the seedlings die. One-half (Su su) are yellow and one-fourth (su su) are dark green (see Figure). The yellow mutant was found to have a normal-to-high rate of photorespiration, but the dark green sister plants had the lowest photorespiration we have so far seen for tobacco. The rates of carbon dioxide uptake by the green and yellow plants are shown in the table together with the rates for Havana Seed, a standard tobacco variety, and hybrid corn for comparison. These and other tests show that the dark green plants are some 20% more efficient in photosynthesis than a standard variety. Now we are trying to establish how this more efficient mechanism is inherited and how to recognize it in a breeding program. Our present tests are laborious and so slow that we can process only a few samples a day.

Ultimately, however, better methods may enable us to screen, as part of a breeding program, for low photorespiration and higher yields through more efficient photosynthesis. Should this genetic manipulation prove effective in tobacco, comparable investigations will be warranted on other crop plants.



Mosquitoes—evidence of spring.

Day Length Governs Seasonal Cycle of Mosquitoes

John F. Anderson
Entomology

Natural site for *Aedes atropalpus.* Jeffrey P. LaFage collecting wigglers from a cavity in rocks along the Housatonic River in Bulls Bridge, Conn.

LIFE HISTORIES of insects in Connecticut, as in other temperate regions, are marked by alternating periods of continuous development and dormancy. Dormancy often persists not only in winter, but also when environmental conditions seem favorable for growth. This dormancy in favorable environments—a sort of suspended animation—is sometimes due to what entomologists call diapause.

Low temperature was formerly considered to be the primary physical factor inducing diapause. Now it is known that photoperiod, or day length, brings on diapause in many insects. Little attention, however, has been paid to the influence of photoperiod on growth of mosquitoes belonging to the genus *Aedes,* our primary pest mosquitoes. My studies of this phenomenon suggest that day length directly governs the seasonal history of at least one mosquito species in Connecticut.

The mosquito selected for study was *Aedes atropalpus,* the rock pool mosquito. It is found from northern South America to southern Canada, but is only locally abundant. Eggs of this species are deposited almost exclusively in rock cavities along rivers and lakes, and the mosquitoes are generally found only in the immediate areas. The rock pool mosquito has three, possibly four, generations a year in Connecticut. In late summer and early fall the numbers of larvae (wigglers) and subsequent adults steadily decline. Only the dormant eggs overwinter and hatch into larvae the following spring.

A puzzling question which I asked at the beginning of this study was why do eggs hatch in midsummer but not in late summer and autumn? To answer this question it was necessary to rear these mosquitoes in the laboratory in controlled temperature and photoperiod environments. Photoperiods of 16 hours and 12 hours per day were selected in the initial experiments because these represented conditions near or just beyond the extremes the species encounters during early summer and fall. Minimum (63°F) and near-

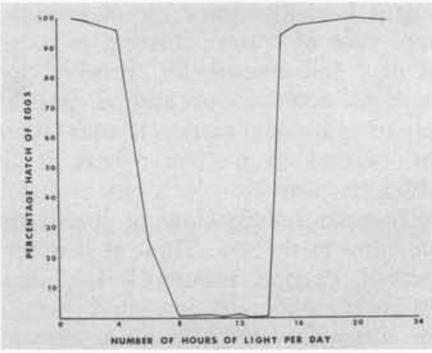
Mrs. Lee Benjamin examining mosquitoes in one of the controlled environment chambers.

optimum (81°F) rearing temperatures were utilized. Results were most revealing. Mosquitoes kept under the "long" day at both high and low temperatures invariably laid eggs that hatched when flooded with the hatching medium. On the other hand, those reared under the short photoperiod deposited eggs that remained dormant. These results clearly disclose that day length and not low temperature determined whether eggs would hatch when inundated.

Once I was sure that the number of hours of daylight was the important external factor in determining hatchability of eggs, I then wanted to know the stage of development of the mosquito which was sensitive to daylight. I determined the critical period by rearing one group of mosquitoes initially under a "long" day and another group under a "short" day. At given intervals of development, mosquitoes were transferred from the long day to the short day environment and vice versa. In all, 10 different experiments were performed. Nonhatchable eggs were produced only in treatments in which the last larval stage and pupa of the parent generation were exposed to a photoperiod of 12 hours. It did not matter what light period young larvae were exposed to, nor did the duration of light during the adult and egg stages affect hatching. Thus the influence of day length operated on the mosquito long before growth was finally arrested. In fact, the sensitive period occurred during the generation preceding the one that entered diapause.

Since diapause is the expression of the short day effect on the parent generation, I wondered whether one or both of the parents were capable of transmitting the "hatchability message" to their sons and daughters. This was easily answered by crossing long-day females with short-day males and conversely long-day males with short-day females. Results revealed that it is the female parent alone which passes this "message" on to the next generation. Long-day females invariably laid hatchable eggs and short-day females deposited eggs that entered diapause.

Thus far I have used the terms short day and long day to refer to day lengths of 12 hours and 16



Duration of daylight as it affects hatching of eggs.

hours respectively. You may now be wondering what are the responses to other day lengths. Mosquitoes maintained at near optimum temperatures were exposed to 14 different day lengths ranging from 1 hour of light to 24 hours. Results of this series of experiments are shown graphically and show that the effect of long days is exerted at photoperiods of 15 hours or more and that of short days at photoperiods of 8 to 14 hours. Surprisingly enough, the long-day effect is also apparent at day lengths of 6 hours or less.

How are all these laboratory findings related to the activity of the mosquito in nature? The critical duration of photoperiod falls between 14 to 15 hours of daylight and functions independently of temperature. Daylight in Connecticut on July 29 is slightly more than 15 hours. By the 25th of August, it has decreased to less than 14 hours. Thus one would expect that larvae completing development throughout July would lay eggs that hatch when flooded with rain or river water. However, female larvae maturing during the latter part of August should lay eggs which remain dormant even when inundated.

Field collection records indicate that the above supposition is true. Larvae are abundant in nature throughout July and August, but become scarce in September. The scarcity of larvae during September is due primarily to the effect of short photoperiods on the late larva and pupa of the female parent during late August and early September. Eggs laid by this late summer brood of mosquitoes do not hatch the same year they are deposited, but instead lie dormant during fall and winter and then hatch when water temperatures rise the following spring. Thus, short photoperiods, not low temperatures, during late summer normally induce diapause of this mosquito. The present study is but one of a series investigating growth of mosquitoes. As we delve further into growth inhibiting mechanisms it is hoped that some of our discoveries in the laboratory will find use in controlling these all-too-often obnoxious pests in the field.

Reducing Evaporation From a Forest

Photo by Fritz Goro, LIFE *Magazine*

Neil C. Turner
Soils and Climatology

Three hundred parts of a simple chemical dissolved in a million parts of water and sprayed on to pine trees growing in a Connecticut forest saved up to 30,000 gallons of water from being lost to the air on each acre of forest. Research, like this being conducted at the Station, is helping scientists to understand and devise better methods of efficiently utilizing the limited amounts of fresh water available to our ever-increasing population.

ALMOST EVERYONE is aware that the rapidly rising population challenges our abilities to produce and distribute sufficient food. And the perceptive reader recognizes that the population explosion challenges just as much our ability to meet the increased demands for water. The world's requirements of fresh water for agriculture, industry, and household purposes are expanding at least as fast as the rise in the population.

We can still increase our food supply by increasing both the area of land in food production and the yield of crops already being grown. There is, however, no way to increase the quantity of water on the earth. It is neither created nor destroyed but used over and over again. Consider the cycle of a single molecule of water. Starting as rain, it may fall on soil, be absorbed by a plant, eaten by an animal, passed on to a human being. It may then be carried to a river where it is used to generate electricity or cool a furnace before flowing hundreds of miles to the sea. There it is evaporated, carried thousands of miles in air currents and deposited as rain in a totally different part of the earth to recycle as it has for millions of years.

Since the amount of water on the earth cannot be increased, and we constantly require more fresh water, we must make more of this water available, increase the efficiency of its use, or both. Desalination of sea water is a promising source of fresh water for industrial and domestic use. Research at this Station is not concerned with desalination but with more efficient utilization of the water available.

At present, rainfall is our only important source of fresh water. This rainfall varies enormously from place to place, from no recorded rainfall over the past 400 years in parts of the Atacama Desert in Chile to more than 500 inches of rainfall per year in areas of Assam, India. In Connecticut the yearly average is a modest but adequate 45 inches, which is reasonably evenly spread throughout the year to give an average of just less than 1 inch per week. At least 70 per cent of this rainfall returns directly to the air in evaporation and transpiration.

The large quantity of water passing from the soil to the air through plants by the process called transpiration comes as a surprise to most of us. In summer, a lawn 33 feet by 33 feet will transpire 600 gallons of water each week; a 1-acre house lot, 27,000 gallons. The forest, which covers two-thirds of Connecticut, transpires roughly 50 billion gallons of water into the atmosphere each week in summer. To produce the grain in a 1-lb. loaf of bread requires 330 gallons of water; the feed to produce a gallon of milk, 3,300 gallons, and the fabric to make a man's woolen suit, 270,000 gallons.

The major proportion of the water used by plants passes through the numerous small pores on the leaves as water vapor. These pores, called stomata, have the ability to open and close. As it becomes dark at sunset, as leaves are heavily shaded by others, or when the water in the soil is in short supply, the stomata close and prevent excessive drying of the plants.

Several years ago Dr. Israel Zelitch of this Station showed that very small quantities of certain chemicals, called antitranspirants, can partially close the openings of the pores. Laboratory and greenhouse tests with small trees showed that this could cause a reduction in transpiration. In 1966, the time had arrived to test the effectiveness of antitranspirants on a larger scale in a forest. A red-pine plantation near Voluntown in eastern Connecticut was chosen for this test. Dr. Paul E. Waggoner of the Station staff and Dr. Ben-Ami Bravdo, a visiting Israeli scientist, arranged for a local tree-sprayer to spray 8 of 16 tracts in the forest with the antitranspirant which had appeared the most promising in the previous trials. The trees were sprayed on June 1.

A close watch was then kept on the level of water in the soil during the next four months. The single spray resulted in a gain of 22,000 gallons of water in the soil under each acre of sprayed forest at the end of this period. Most of this accumulated during the first month.

A similar experiment was conducted last summer. Under the wetter conditions of 1967 and by spraying the trees in both June and July, we were able to prevent 30,000 gallons of water from escaping into the air. Using a porometer, an instrument which measures pore size, we were able to show that, as predicted from laboratory studies, the saving was the result of a partial closure of the leaf pores during the daytime. On a forest-covered catchment basin, much of the water so conserved could be expected to increase the input into the reservoir during the succeeding months.

Since the sun usually dries trees faster than they can obtain water from the soil, their needles dry and their trunks shrink as water is withdrawn. A micrometer depth gauge showed that by early afternoon on sunny days, the diameter of the tree trunks was a few thousandths of an inch less than in the early morning. Spraying prevented the needles from drying and building up a water deficit as great as in the unsprayed trees. This caused the trunks to contract less, indicating that the partial stomatal closure had saved water.

Insufficient time has elapsed to determine what long-term effects the spray will have on tree growth. We know that there was no effect on growth in 1966. During 1967, radial growth of the tree trunk was reduced to two-thirds that in the untreated trees, but total growth was less affected. As timber is generally only a by-product in catchment areas of watersheds, this reduction is not considered to be very serious when compared to the increase in the stream flow to the reservoir.

The results of this Station's research have wider application, especially in areas where droughts are more frequent. I immediately think of the southeastern region of South Australia where I was a graduate student. A forestry industry has been established in this region where the average annual rainfall is from 25 to 30 inches. This rainfall occurs principally in the winter months of June, July, and August. In years such as 1967 when rainfall is less than 15 inches, the tops of the trees die back because insufficient moisture is available from the soil during the hot, dry summer months. Spraying with an antitranspirant in September or October could retain sufficient moisture to prevent this deterioration of the timber.

We are a long way from being able to regulate water loss from all crops at will, but the research already undertaken is helping the scientists to understand the important role that the micropores of the leaf play in transpiration and growth of a crop. As a result, the possibility of supplying the demands of future generations for water is a little nearer.

Dr. Paul E. Waggoner determines soil moisture in the treated area of the forest.

Precise Analytical Steps Give Consumer the Facts

J. Gordon Hanna
Analytical Chemistry

THE GOAL of chemical analysis is information for a purpose. The purpose of the information obtained by analytical chemists at this Station is to protect the Connecticut consumer. The buyer is becoming more knowledgeable and more demanding that his purchases meet his expectations. Increasingly the consumer voice is being heard and heeded in the legislative halls. Good administration of the consumer protection laws depends on a sound basis for decision making, and this is provided to a large extent by analytical information.

Products officially analyzed at this Station for regulatory purposes include foods, beverages, drugs, cosmetics, animal feeds, fertilizers, pesticides, hazardous substances, and milk for vitamin contents and for pesticide residues.

The job of any analytical chemist can be divided arbitrarily into five operations: Procurement of a representative sample, selection of an analytical technique, conversion of the desired constituent into a measurable state, measurement of the desired constituent, and calculation and interpretation of the data in terms of information desired. How Station analytical chemists perform is here reviewed in terms of this series of operations.

Official samples of commercial feeds, fertilizers, and pesticide formulations are collected by the Station inspector. The feed and fertilizer laws are administered by the Station Director and the pesticide law by the Commissioner of Agriculture and Natural Resources. The Department of Consumer Protection inspectors collect and submit samples of foods, drugs, cosmetics, and samples to be checked for compliance with the hazardous substance law. The Connecticut milk supply is sampled by inspectors from the Department of Agriculture and Natural Resources. Other state agencies submit samples to check compliance with state purchasing requirements. The original samples are selected at random in the marketplace. It is then the analytical chemist's responsibility to be certain that the portions taken for the analyses are representative of the complete sample. Either the total sample, if practical, or a statistically selected subsample is brought into a homogeneous state by grinding or mixing and portions used as the analytical samples.

The analytical chemist must have a broad understanding of the various techniques available to be able to select the one which will yield the desired information in the most efficient manner within the limits of accuracy and precision specified. Standard methods are useful only in narrowly defined situations. More often than not in view of the variety of samples encountered, special adaptations of the available methods are necessary. Often it is impossible with the information at hand to select with confidence a single method or technique. Then the worker tests several approaches using standard materials, the composition of which approximates that of the sample.

Conversion of a constituent into a measurable state is probably the area in which the analyst's ingenuity is taxed most. This is especially true in the isolation of small or trace amounts of a material from different chemical environments. This type of analysis is becoming more common. Modern food technology has resulted in the increasing use of trace amounts of additives such as vitamins, minerals, preservatives, flavors, and colors. The types and limits of these materials permitted in foods are strictly detailed by law and their analyses are necessary for regulatory purposes. Medicants, minerals, and vitamins are added to animal feeds in guaranteed amounts, and analyses are made to see that the guarantees are met.

Pesticide residue analysis of fruits, vegetables, milk products, and animal and fish tissues is a common example of a trace determination—

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often in the parts per billion range.

Also in many cases the concentration of an ingredient in a prepared food is evaluated based on the determination of a minor constituent characteristically present in the ingredient. Thus the technique actually involves the isolation of a trace component. An example of this is the estimation of the egg solids content of bread, noodles, or ice cream based on the determination of lipoid phosphorus. The lipoid phosphorus is usually present in 0.2 per cent or less if egg was actually used in the preparation process. Other examples are the estimation of juice contents of fruit beverages from determination of ash, phosphorus, and potassium concentrations and the estimation of milk solids in various prepared foods from lactose determinations.

Some sort of solvent extraction is usually involved in the isolation procedure. In this connection conversion of the component into an extractable entity is often a prerequisite. For example, salts are converted to acids for extraction with organic solvents. The most useful and often the only successful methods for trace components are those based on some type of chromatography—gas, column, paper, or thin layer.

The measurement step, once the original analytical technique has been selected and the isolation successfully accomplished, is normally routine but requires experience and often specialized technical ability. It may involve, for example, a chemical titration, a weight determination, the measurement of a physical property such as light absorption, flame or spectrographic emission, or an electrical property, or observation of the physiological effect of a material on test animals. Gas chromatography, in addition to being an isolation technique, is also valuable, after proper standardization, for the quantitative determination of trace materials. In this, as in all the steps, close observation is maintained to spot any abnormalities which may indicate deficiencies in the method or the presence of interferences which may call for investigations.

An analysis is not complete until the results have been expressed in such a manner that the person for whom the results are intended can unequivocally understand their significance and relate them to the purposes for which the analytical data were requested. The analytical chemist is responsible for the interpretation of the data because he is fully aware of the limitation of the processes of the analytical procedure.

Our analytical results are reported directly to the agencies requesting the work and—for feeds and fertilizers—to the manufacturers. In addition, for the benefit of the consumer, annual bulletins are published containing data obtained for commercial feeds, commercial fertilizers, commercial pesticides, and for official food, drug, and cosmetic samples. Computer processing to speed the calculation and dissemination of the analytical results is being initiated.

Because of the variety of problems which confront the analytical chemist, he is constantly facing challenge and surprise. He alters and improves his established procedures to make them applicable to special situations and searches for new principles to advance the science of chemical measurements. The resulting improvements and discoveries are passed along as better service to the consumer.

Sullivan to Speak on Science at Work Day

Walter Sullivan

Walter Sullivan, science editor of *The New York Times,* will be the principal speaker on the Science at Work program to be presented at Lockwood Farm in Mt. Carmel, Wednesday, August 14.

He will deliver the Samuel W. Johnson Memorial Lecture at this 56th summer field meeting staged by the Station in Mt. Carmel.

Mr. Sullivan, a Connecticut resident, joined the staff of the *Times* as a copy boy in 1940 after his graduation from Yale University. After World War II service as skipper of a converted destroyer, he rejoined the *Times* in 1946 as a correspondent.

Reporting from China, he was one of the last outsiders to cross the Gobi Desert and visit Sinkiang before the Communists marched in. He covered the Korean War and the "cold war" between Russia and the West in the struggle for Germany.

He participated in Antarctic expeditions in 1954 and 1956 and was in charge of the special *Times* coverage of the International Geophysical year. His coverage led to the George Polk Memorial Award in 1959.

Mr. Sullivan is the author of "Quest for a Continent," "Assault on the Unknown," and "We Are Not Alone," 1965 winner of the International Non-Fiction Book Prize. His newspaper writing on the natural sciences was recognized in 1963 when he received the annual award of the American Association for the Advancement of Science—Westinghouse Educational Foundation.

Mr. Sullivan will speak at 1 p.m. The program of talks by Station staff members, discussions, and inspection of research plots and exhibits begins at 10, continuing to 8 p.m. Dr. Lester Hankin of the Department of Biochemistry is general chairman of Science at Work.

Gypsy Moths Respond to Crowding

David E. Leonard
Entomology

The gypsy moth needs no special introduction to most readers of this journal. Since the gypsy moth first invaded Connecticut at the turn of the century, many have seen wooded areas denuded by this pest.

Gypsy moth larvae are innocuous when they are few, for they remain secluded during the day. Hordes of gypsy moth caterpillars, however, provide an unpleasant experience for people who have chosen the tranquility of the woods for their homesites, because when larvae are crowded they are active both day and night, crawling on and over everything they encounter. This and other observed differences in sparse and crowded populations of gypsy moths prompted my research on the effects of crowding.

The number of gypsy moths varies widely from year to year and from place to place. The insect's fate can be predicted when it occurs in great numbers, for mass outbreaks are followed by population crashes. The fate of sparse populations, however, cannot yet be predicted. These populations can (1) decline to extinction, (2) remain stable, or (3) burgeon and become foci for epidemics. Immediate control measures are necessary only for the populations which will burgeon.

It seemed evident when comparing sparse and dense populations in nature that the rate of development to adulthood was slower in sparse populations. If the rate of development is influenced by numbers of larvae, this would not only be of interest biologically, but would also be of prime importance in the timing of possible new methods of control, such as the release of sterilized males.

Many variables influence the rate of development, thus to study the effects of crowding in nature is difficult. Conditions can be controlled in the laboratory, however, and the variables reduced to one, crowding. Laboratory studies necessitate feeding gypsy moth larvae, a difficult feat with a leaf-eating insect. Several years ago we devised a synthetic food. Although it looks more like cheese than leaves, the gypsy moth larvae thrive on it, and we now study this insect in the laboratory throughout the year.

An experiment was designed to compare larvae grown under crowded conditions with larvae reared individually. Pupae and adults were not crowded. These studies showed that crowded larvae developed to adulthood faster, confirming the observations in nature that crowding hastened the rate of development. Surprisingly, the crowding of larvae affected the rate of development differently during different stages of growth. Development was slowed during the first larval stage. All other stages developed faster, including the pupal stage, even though all pupae were kept individually in small cups. The faster rate of development of pupae from larvae which were crowded indicates that events which occurred earlier in the life of the insect could, probably through hormonal action, influence the rate of development later in the life of the insect. This was shown in yet another way, for the adults from crowded larvae were lighter in color than those from larvae reared in isolation.

Another aspect of Dr. Leonard's investigations on the biology of gypsy moths is reported in "Differences in Development of Strains of the Gypsy Moth," Station Bulletin 680, available on request.

Crowding also induced larvae to undergo additional molts. In order to grow, larvae must shed their skin, a process known as molting. Normally, males molt four times before pupating; females, five times. Some larvae undergo additional molts, and these larvae have been of interest, for their biology is different in several important ways. They have a longer period of development, mostly because they pass through an additional larval stage. They feed more, hence they grow larger, and the females lay more eggs. They have a shorter pupal period, and perhaps this would reduce the amount of predation and parasitism during this vulnerable stage. Any of these factors could influence the success of these types of larvae in a population; the effect on the number of eggs being of singular importance.

Larvae with an additional larval stage also have a longer first larval stage, the stage during which this insect spreads from one area to another. In insects, normally the adult stage is important for dispersion, but gypsy moth females cannot fly. When gypsy moth larvae emerge from the eggs, they are tiny, covered with long hairs, and are readily carried by the wind. We now know that both crowding and hunger cause larvae to become more active. The movement of a branch caused by a breeze can induce these larvae to drop and hang from a thread of silk, providing a greater opportunity for the wind to pick up the larvae and blow them away. This is an interesting example of the evolution of a mechanism for dispersal which operates during periods of larval stress, thus providing more opportunity for larvae to leave an unfavorable habitat.

The wind can carry gypsy moth larvae for many miles. With their fate literally left to the winds, few larvae are deposited where they can survive. The survivors will grow larger, and larger females lay more eggs. The increase in egg-laying capacity helps offset the loss of the great numbers of larvae deposited in habitats where they cannot survive.

Within each larva are the genes, which, through the millenia, have enabled its ancestors to adapt to many new environmental conditions, both favorable and adverse. The insect that successfully adapts to its new environment will pass its complement of genes to its progeny. Each dead insect eliminates from the total pool of gypsy moth genes that combination of genes which failed to survive the rigors of natural selection.

What factors increase or decrease the gypsy moth's chances for survival? Are some of these factors expressed in morphological or behavioral differences which can be observed in nature? If so, they will provide a key to the predictability of population trends. These are but some of the mysteries enveloped in the skin and scales of the gypsy moth.

Activated Carbon Adsorbs Pesticides

John F. Ahrens
Plant Pathology and Botany

James B. Kring
Entomology

Activated carbon, sometimes called the "universal antidote," is perhaps best known because of its widespread use in cigarettes and in water purification. In agriculture, new uses are still being discovered for this highly adsorbent material. It has been used to clean spray tanks, to protect plants from insecticide and herbicide injury, and to prevent off-flavor in food-plant crops from pesticide residues. Activated carbon is also used as a seed coating, both to protect the seed from a pesticide and to lengthen the effective life of the pesticide.

Research at this Station during the past 5 years has shown practical ways to inactivate several different kinds of pesticide residues in soils. In studies with a range of organic herbicides, many were found to be readily adsorbed and hence detoxified by activated carbon applied at rates of 50 to 400 times the amount of the herbicides in the soil.

Included in these tests were several persistent herbicides such as monuron, atrazine, simazine and bromacil, and crabgrass killers such as DCPA, bensulide, and DMPA, commonly used on home lawns. We have found that reseeding on lawns treated with any of these herbicides can be successful when activated carbon is first worked into the soil surface.

The results are not all positive, however. Highly water-soluble and poorly adsorbed herbicides, such as dalapon, may not be readily detoxified by carbon treatment and highly active herbicides, such as picloram, can be detoxified only with very high rates of carbon application. Long-term studies showed that once a crop was protected from an herbicide by carbon treatment of the soil, successive plantings of the same crop also were protected.

Experiments also showed that the

Misuse of an herbicide killed the grass in the background.

uptake of chlordane and heptachlor residues in soils by carrots, potatoes, and radishes could be reduced, but not eliminated, by treating the soil with activated carbon. Low residues that have persisted in the soil for a long time are more difficult to counteract. However, we now have a good deal of information that is useful in solving problems caused by changes in cropping systems. These problems arise when pesticides applied on one crop persist and are taken up by crops grown the next year.

Further experiments in zonal or root-dip treatment with activated carbon have revealed several possibilities for safening the use of herbicides in transplanted crops, for example strawberries and conifer seedlings. Root dips in both dry powdered activated or 15 per cent slurries (1¼ lb. in 1 gal. of water) protect these plants from herbicides applied before or after planting and thus give efficient weed control at low cost.

We have been able to develop simple techniques for determining whether soils are contaminated with plant damaging levels of pesticides. Satisfactory answers to problems involving soil contamination by accidental use or misapplication of pesticides in home lawns, orchards, golf courses, crop plants, and greenhouses have been obtained as a result of this research.

As We Were Saying

Quotes on Plants, Insects, and People From the Pages of Frontiers

1954 **The Insect Resistance Problem**
The use of insecticides is to an economic entomologist the last step, to be taken only after all other methods of control have been found wanting. The classical approach to insect control has always been, first, to study the life history of the pest, with the hope of finding some way to avoid its damage. If that is unsuccessful, the second approach is to study the parasites, predators, and diseases of the insect. If these offer no relief, the use of insecticides to prevent serious economic losses is considered as a third resort. *Neely Turner*

1955 **From the Director**
During the past 30 years, we have gained for our own food some 65,000,000 acres of land formerly used to feed the horses that provided the power when horsepower came from horses. If every horse left were converted to glue, however, the land gained would not equal that spent on the New Jersey Turnpike. *James G. Horsfall*

To the Editor
Frontiers of Plant Science
Dear Sir:

May I thoughtfully express my opinion of your publication, Frontiers of Plant Science.

Having delved for thirty years, searching for the truth, your publication comes as one unique, in that it is condensed, concise, and covers a practical point of view of material which is most sought by the layman, in the most thought of fields.

To you, and the others with you in this medium, thanks, and thanks evermore.

Sincerely,
JOSEPH BOSILEVAS

Stamford, Conn.
December 15, 1960

1957 **Wisdom Is Better Than Rubies**
Within the past year a new method has been developed whereby isocitric acid can now be fairly easily isolated from leaves and prepared in the form of a beautifully crystallized potassium salt that is a new compound never before known. It is impossible to place a monetary value on such a substance. . . . the small bottles of isocitric acid in the Department vault are treated as if they were filled with rubies. *Hubert B. Vickery*

1957 **A Forest Is More Than Trees**
The practical results of research help our efforts to keep the forest productive. The discovery of how the forest system "works" helps us and our children appreciate the significance of the woodlands we preserve to enjoy. Finally our thoughts on the complexity of the woods remind us of more far-reaching lessons: that mankind's welfare is bound inescapably to the integrity of the web of relations which nature has woven into a balanced and organized system; that many kinds of research are needed to show how the threads intertwine; and finally that, when man slashes into the web blindly, the unbalance in nature's system may literally bring unexpected troubles to his own front door. *Jerry S. Olson*

1959 **We Face Uncomfortable Decisions**
Our way of life is changing, whether we like it or not. Our struggle for existence and our competition, even among ourselves, are becoming more complicated. More and more uncomfortable decisions and choices will have to govern our actions, for nature is not a personal friend of ours, regulating its creatures in numbers to suit us. *Raimon L. Beard*

1964 **New Ways to Repel Aphids**
As one might expect, I then found that plants surrounded by aluminum, by orange-colored plastic, and to a lesser extent by shining black plastic were protected from attack by aphids. These results suggest many practical applications. It seems conclusive that by simply altering the reflectance and the color of the background of plants we can induce flying aphids to move along and leave our crop plants unmolested. *James B. Kring*

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.

Entomology

B684 *Control of Lace Bugs on Broadleaf Evergreens.* John C. Schread.

B693 *Leaf Miners and Their Control.* John C. Schread.

Reports on Inspection

B682 *Pesticides, Report of Inspections, 1964, 1965, and 1966.* J. Gordon Hanna.

B683 *Commercial Feeding Stuffs Report, 1965.* J. Gordon Hanna.

B685 *Commercial Feeding Stuffs Report, 1966.* J. Gordon Hanna.

B690 *The 70th Report on Food Products, 1965.* J. Gordon Hanna.

B691 *The 71st Report on Food Products, 1966.* J. Gordon Hanna.

B692 *The 72nd Report on Food Products, 1967.* J. Gordon Hanna.

C229 *Commercial Fertilizer Inspection Report, 1966.* J. Gordon Hanna.

The following *Food Products* inspection reports are also available: B688, 1963; B689, 1964.

Robert Josephy

A. Ward Spaulding, Vice-president of the Board of Control, 1955 to 1967.

Josephy Elected Vice-president of Board

Robert Josephy, Bethel, was elected vice-president of the Station Board of Control on October 26, 1967. He succeeds A. Ward Spaulding, Suffield, who continues as the governing board member named by the University of Connecticut. Dr. Ross A. Gortner, Jr., of Wesleyan University, replaces the late Dr. Edmund W. Sinnott as secretary of the Board. Ellis C. Maxcy, North Haven, is now the Yale University appointee on the Board. Mr. Maxcy was president of the Southern New England Telephone Co. at his retirement last October.

Other members of the Board of Control are Governor John Dempsey, president; Joseph N. Gill, Commissioner of Agriculture and Natural Resources; Thomas M. Burgess, Wapping; and James G. Horsfall, Station director.

Twenty Years

(Continued from page 16)

have to assure that America never runs out of food. I have seen hunger stalk the land in India, and that must not happen here.

On the other hand the same population explosion is creating and will continue to create severe stresses on our environment. And so we must now move further and faster to put our science to work on the effect of man on his environment.

As plant scientists we can play a unique role. Plants are the great purifiers of our environment—plants that range from the bacteria in a sewage system to the stands of trees that help to purify the air. Green plants can be adapted, I think, to purifying sewage too.

We can only keep rolling back the layer of ignorance at the frontiers of knowledge, watching carefully all the time for the nuggets that may be uncovered. This policy has paid regular dividends to Connecticut citizens for almost a century now. I know we can improve the acuity of our vision with mass spectrometers and gadgets for measuring nuclear magnetic resonance, but no gadget can ever substitute for imaginative young scientists with a yen to know and understand.

It is to them that we must entrust the next 20 years in the life of this journal, for theirs is the challenge to find new knowledge. I can already see a lead opening ahead. Crowding will worsen as a factor in human society. One young man, Leonard, reports to you in this issue on the effects of crowding on the reactions of gypsy moths in his laboratory. He is only on the threshold. He can study the effect of crowding on their nerves, their digestion, their instincts—many things. What he learns should be useful to sociologists.

And so I join the ranks of the frustrated forecasters. I think that we at the Station have changed our course significantly during these 20 years of transition. We have broadened our traditional concern with the influence of the environment on man to include a deep concern with the influence of man on his environment.

It has been said that nature extends credit freely, but always sends a bill. Enlightened research and a concerned citizenry can help us meet the installments now overdue, and put our environmental management account in better order. The challenge to our staff is to continue to provide the required research.

Twenty Years of Frontiers

James G. Horsfall
Director

WITH this issue, FRONTIERS OF PLANT SCIENCE completes its first two decades of reporting our science to the citizens of the state. Next year it becomes 21 years old.

What better time to relive the 20 years and to steal a peek at the future? What was science and technology like 20 years ago? Television sets were rare, and no commercial passengers flew in jet airplanes. The whine of the power mower had not yet disturbed the serenity of suburbia.

Here at the Station, Morgan's soil test was little more than 10 years old. Hybrid corn had attained real prominence only a few years before. We had just learned how to grow potatoes free from wireworms, while our zineb series of fungicides was only 4 years old.

Now Morgan's soil test or variants of it are used on most agricultural soils of the world. The Deccan Hybrid variety of corn is helping India to its agricultural feet. Nobody ever hears of wireworms on potatoes. Zineb is used on most of the world's potatoes to prevent the dread late blight disease and on most European vine grapes to prevent the downy mildew disease.

I think we can say that prior to the publication of Volume 1, Number 1, our basic aim was to conduct research on the effect of environment on man—especially, of course, how he could procure the food he needed from his environment, and how he could deal with the gypsy moths, the mosquitoes, and the roaches and termites that pester him.

During these 20 years, we have continued to report researches of the effect of environment on man: a charcoal kiln, chemotherapy of plant disease, hybrid corn without detasseling, hybrid chestnuts, wood preservatives, sewage sludge for fertilizers, root growth and soil compaction, the suburban forest, new ornamentals, nematodes on crops, nuisance insects in homes and backyards and estuaries, and purity of foods for sale.

During these 20 years FRONTIERS has also reported to you the discovery of the biochemical base for the opening and closing of the stomata on leaves. Stomata are the gates through which pass inbound all the carbon dioxide to produce the world's food and through which pass outbound two-thirds of all the water that falls on vegetation-covered soil. We report to you in this issue on ways to control stomatal movement, thus to save water for reservoirs here and perhaps worldwide.

And so on.

But during these 20 years, we began to investigate the effects of man on his environment—a new direction for an agricultural experiment station. As early as 1949, when this journal was a baby only one year old, we began to study the green growth scums on Lake Zoar. We quickly found that the plants of the green scums were growing in Lake Zoar on the fertilizer elements brought into the lake from treated sewage—the better the sewage treatment the more the green growth.

Later we discovered "weather fleck" on tobacco, a mysterious disease soon shown to be due to the ozone formed in the air from automobile exhausts. We now have two investigators working full time on the effects of auto exhaust gases and other pollutants on plants.

Still later we had to move into the problem of de-icing salt and its effect on roadside plants.

Where next? Where next indeed. We must always try to look far down the road ahead. In the face of a population explosion, we dare not quit research on the food supply. It is surely our responsibility to do all that we can with the facilities we

(Continued on page 15)

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BRUCE B. MINER, Editor

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James G. Horsfall
Director

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