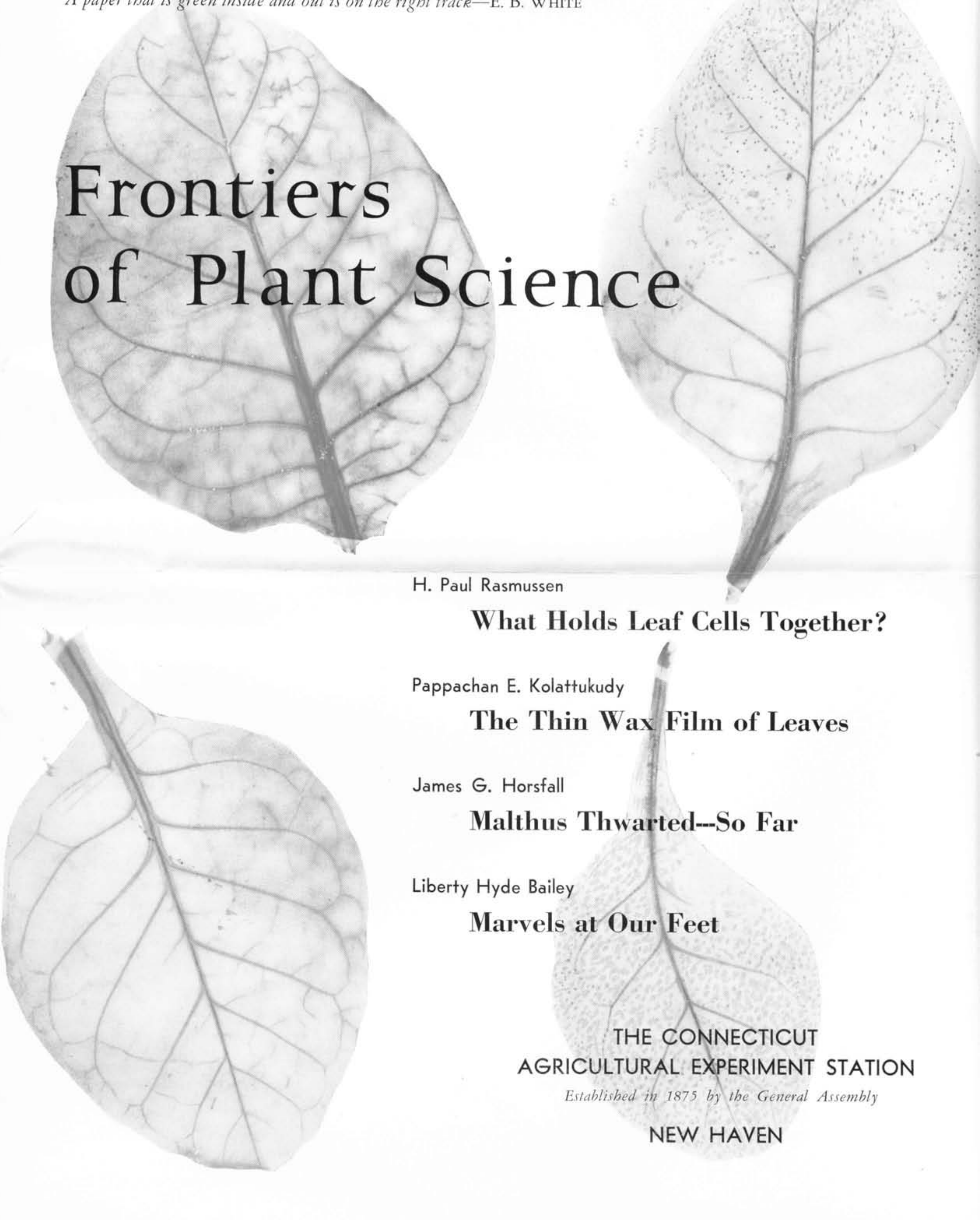
A paper that is green inside and out is on the right track—E. B. WHITE

SPRING 1966



Frontiers of Plant Science

H. Paul Rasmussen

What Holds Leaf Cells Together?

Pappachan E. Kolattukudy

The Thin Wax Film of Leaves

James G. Horsfall

Malthus Thwarted--So Far

Liberty Hyde Bailey

Marvels at Our Feet

THE CONNECTICUT
AGRICULTURAL EXPERIMENT STATION
Established in 1875 by the General Assembly
NEW HAVEN

The world is young . . .
the sunshine good.

Marvels at Our Feet

Liberty Hyde Bailey

INCONCEIVABLE are the energies locked in the unyielding earth and the invisible air! Flowers and herbage all perfect and shapely and colorful, wrought by the countless addition of cell on cell, are whipped away, broken by storm, trampled by cattle, as if they were the very riffraff of creation.

Numberless millions of insects — matchless in shape, complete to the last detail, with an amazing exactness and adjustment of circulatory, digestive, nervous and sexual systems — dance for a moment in the sun, then are gone forever. And no account is kept. The millions of men have come hopefully into being and have forthwith passed away; and nobody knows where they have gone. The legions rise, march their little day, and perish. Others take their place. The pageant never halts.

See now how these leaves of this small strawberry plant stand forth extended to bathe themselves in light. Delicate, invisible forces of life are moving mysteriously in these thin tissues, hidden away in laboratories themselves invisible, every laboratory and every process as perfect and fully furnished as if each leaf were to last forever.

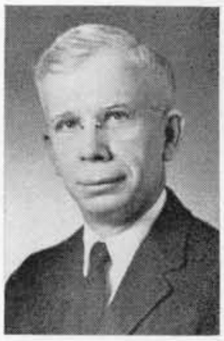
These leaves will die. They will rot. They will disappear into the universal mold. The stuff that is in them will pass elsewhere, perhaps to the egg of a newt, to the root of a tree, to a fish in a pool. The energy will be released to reappear, the ions to act again, perhaps in the corn on the plain, perhaps in the body of a bird. The atoms and the ions remain or resurrect; the forms change and flux. We see the forms and we mourn the change. We think all is lost; yet nothing is lost. The harmony of life is never ending.

Every man knows in his heart that this is so. Every man knows in his heart that there is goodness and wholeness in the rain, in the wind, the soil, the sea, the glory of sunrise, and in the sustenance we derive from the earth.

We deceive ourselves if we turn from the essentials and try to satisfy ourselves with the small and trivial gratifications of this age. Let us look more closely about us and see how good are the common things, how marvelous are all things made at the beginning. The meaning of life is in its beauty. And ten thousand years from now children will call across the centuries that the world is young, that the sunshine is good, that love and faith, and mystery and the buoyancy of life are the only realities.

Marvels at Our Feet *is an excerpt from the article with the same title in* Forever The Land, *edited by Russell and Kate Lord, copyright 1950 by Harper and Brothers and here reprinted by permission. The young lady, photographed at Lockwood Farm of this Station last August, is Cathy O'Donnell of Hamden.*

Malthus argued a century and a half ago that man, by using up all of his available resources, would forever press on the limits of subsistence, thus condemning humanity to an indefinite future of misery and poverty. . . . The truth or falsity of his prediction will depend now, with the tools we have, on our own actions, now and in the years to come. JOHN F. KENNEDY



James G. Horsfall

Malthus Thwarted--So Far

James G. Horsfall, Director

THE YEAR 1965 brought a great shift in American thinking on food and agriculture. In that year the name of Malthus rang out loud and clear. It had been muted before. In fact a year or two earlier an article in *Newsweek* had mentioned the "outmoded Malthusian doctrine."

Before 1965, we Americans had lived complacently with the comfortable feeling that we were immune to worries about food. The bursting Butler bins of the grain belt guaranteed that, we thought.

But in January 1965 a new book appeared, "The Hungry Nations," by Paddock and Paddock. We stirred uneasily after a big Sunday dinner as we read the review in *The New York Times*. Was the world *that* hungry? It was.

We checked and discovered that our bins were being emptied at a rapid rate to feed the hungry nations that the Paddocks had described. Our surplus supplies of dried milk, dried eggs, and cheese were nearly gone. Corn was low, and even wheat was down to 1½ years supply for just us, not for us plus the rest of the world.

Our unease intensified as the year wore on. Come summer we discovered that India was in the middle of the worst drought in a century (it continues in 1966) and that food riots were occurring. We remembered the last great famine in India in 1943. It filled our papers in spite of the war.

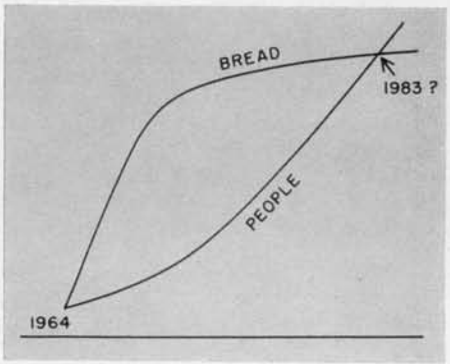
Was it possible, we wondered, that our "seven fat years" were drawing to a close? Was it possible that the "seven lean years" were somewhere near? Could we feed millions of Asians and Africans and others as well as ourselves?

When 1965 ended, we found that the world had not produced more food than in 1964, but it had produced 60 million more people. This is equal to adding a third of the population of the U. S. Although we greatly increased our food shipments, someone somewhere ate less food in 1965 than in 1964.

The effect of fateful sixty-five can be found in several places. It appears in the opinions of the editors of *Life*. Earlier their editorials had complained about the "farm mess" and the folly of surpluses. A few weeks after the end of sixty-five a new editorial appeared, "The Warning of India's Famine." The editors said *inter alia,* "It is true that the gloom of a whole line of Malthusian prophets has always — so far — been thwarted by man's ability to improve his environment."

The magic words in this piece are *so far*. A note of doubt has crept in. The editors of *Life* must have seen the graph for grain production-food needs published by the U. S. Department of Agriculture. To me, this graph shows that the curves for the production of food in the world and the production of people in the world are on a collision course. The curves run together head-on by the early 1980's — unless something drastically alters the course of one or the other, or both.

(continued on page 8)



One aspect of the world food problem. The PEOPLE line represents the food aid needs of 66 developing countries. The BREAD line represents grain that the United States arsenal of agriculturalization could supply. *Adapted from information published by the Economic Research Service, U. S. Department of Agriculture.*

What Holds Leaf Cells Together?

Calcium Pectate, Says a Histochemist

H. Paul Rasmussen

Autoradiogram of radioactive calcium in a tobacco leaf.

LEAVES are some of the most abundant objects around us, both winter and summer. Many of the chemical reactions taking place within a leaf are well known, but the question a small boy might ask on a walk through the woods has gone unanswered: "What holds a leaf together?"

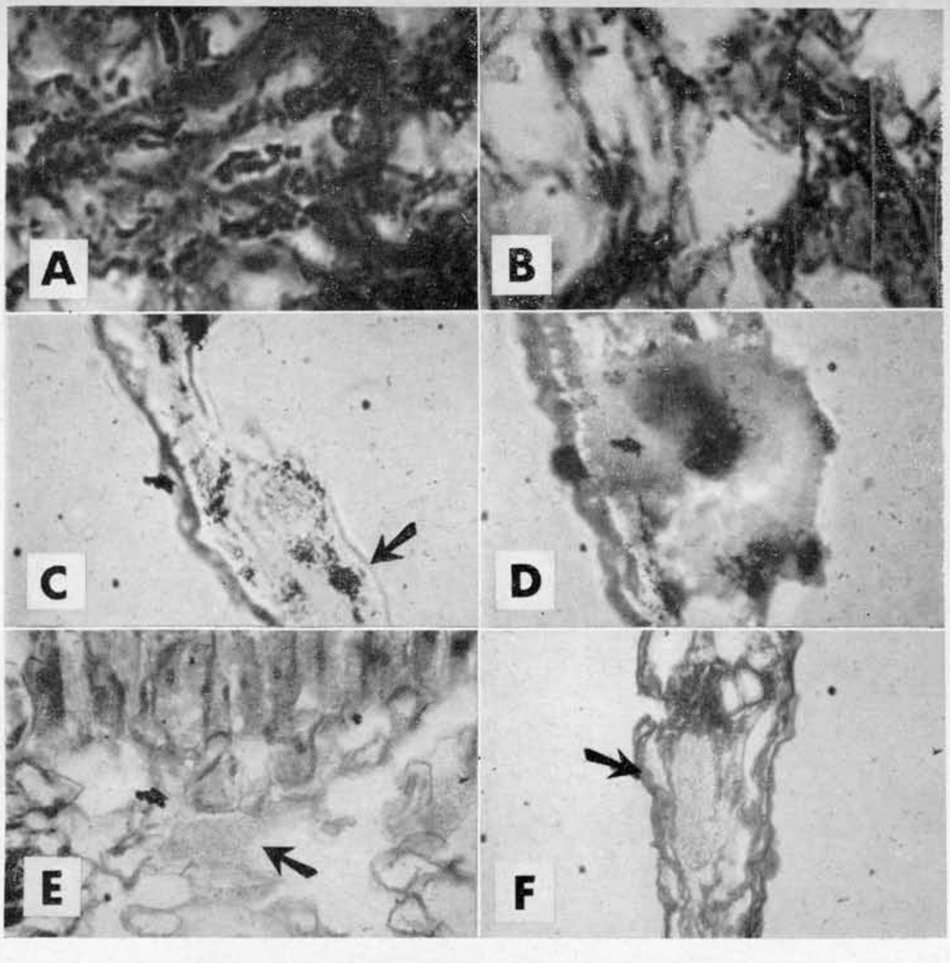
During the course of a growing season it is evident that all plant cells are not held together with the same strength. For example, plant parts can be seen falling from early spring to late fall — petals from flowers, and seeds, fruits, and leaves from all types of plants.

Obviously these cells which separate are not held together as strongly as neighboring cells. Correspondingly, we can assume that the strength of the bonds between cells varies from plant to plant, especially when grown under varying environments.

Of the plants grown in Connecticut, tobacco is clearly one in which leaf strength influences leaf quality. For this reason I used tobacco as an experimental plant.

Some tobacco leaves, after fermentation, are weak and rupture easily. Microscopic examination of torn leaves revealed that a weak leaf separated between cells, whereas a strong leaf tore through cells. This indicated that the amount or form of pectin, the material between the cells, must be abnormal in the weak leaf. Unfortunately for the hypothesis, histochemical determinations indicated no difference in the quantity of pectin or of cell wall constituents. However, in weak leaves I found pectin in a form different from that in strong leaves, and the hypothesis was sustained. That is, when very thin (10-micron) sections were cut and the calcium located in both the strong and weak leaf, I found that a strong leaf (photo A) had approximately 10 times more calcium than a weak leaf (photo B). Calcium is identified by the black spots. Thus inadequate calcium is apparently responsible for the difference in leaf strength. This is logical because calcium joins with pectin to form a binding material or biosynthetic cement between adjacent cells.

If lack of calcium is responsible for weak leaves, then adding adequate calcium should form "bridges" between pectin chains, cement the cells together, and so strengthen the leaves. Conversely, extracting the calcium or replacing it



How calcium is identified in tobacco leaf tissue. Photograph A shows a strong leaf, B a weak leaf. Black dots in C show distribution of radioactive tracer calcium within the tissue of a tobacco leaf. The arrow indicates calcium in the crystal. Microautoradiogram D shows addition of calcium to an existing crystal. A calcium crystal in the living leaf is shown in photo E, and in a fermented leaf, F.

with other elements which do not form these bridges should weaken strong leaves. Adding calcium to weak leaves did make them as strong as normal. Displacing calcium with sodium did make normal leaves weak.

A seeming paradox led us to trace the calcium that entered the plant. The paradox was this: while less calcium was found in the pectate cement of weak than of strong leaf sections, an equal amount of calcium was found in the analysis of both entire leaves. Using ^{45}calcium, a radioactive tracer, and making autoradiograms of the plant (see cover photo) we showed that calcium moved into all the leaves. Most surprising, however, were the small isolated spots of radioactivity visible between the leaf veins (top photo, p. 4). When microautoradiograms were made of 10-micron-thick sections of the leaves, these isolated spots of radioactivity were found to be crystals. These crystals or grains may be easily seen in fermented Connecticut shade-grown tobacco (photo F) and they are also present in green leaves (photo E). The ^{45}calcium not only formed entire crystals (photo C, arrow) but also added to existing ones (photo D). Of course, some ^{45}calcium formed the calcium pectate bonds between cell walls (photo C, black dots except large spot at arrow).

The existence of ^{45}calcium in at least two places, crystals and between cell walls, partially explains the paradox of less calcium between cells of weak leaves while the total calcium content of entire weak and strong leaves is equal.

We have found that the crystal content in the weak leaf is greater than in the strong leaf. Calcium in crystal form is not available for forming the pectin bridges or bonds that strengthen the leaf. An additional explanation of the paradox is provided by analysis of a water extract of a strong and weak leaf. Thirty per cent more calcium was extracted from the weak leaf than from the strong, indicating that less calcium was bound to the pectin in the tender leaf.

Neither the crystalline nor the water-soluble calcium are detected in the histochemical test (photos A and B), but both are detected in the quantitative analyses of the entire leaves. Neither the crystalline nor the water-soluble forms are in the calcium pectate bonds that hold leaves together. Clearly, the amount of calcium in the form of calcium pectate is less in the weak leaf, and therefore its leaf cells separate easily.

Having found that calcium pectate holds leaf cells together and having used calcium to strengthen a weak non-living leaf, I felt that the same thing could be accomplished in the field by increasing calcium uptake. I found that plants grown on acid soil, unfavorable for calcium uptake, had weaker leaves than those grown on soil near the neutral level of pH 7.

In both laboratory and field we have been able to control leaf strength. The amount of calcium which forms the pectin bridge between cells depends on the form of calcium within the leaf and on the presence of elements which interfere with its formation.

Being able to increase leaf strength in the field is important not only to tobacco growers, but to others as well. We may have taken a step toward making it possible to protect other crop plants and ornamentals from damage by wind and hail.

Science at Work

Annual harvest of new knowledge produced by research at The National Historic Landmark Experiment Station. All interested are invited to Lockwood Farm in Mt. Carmel.

Wednesday, August 10

Lockwood Farm

News Notes

A parasitic wasp, *Brachymeria intermedia,* that kills gypsy moths in the pupal stage is now established in at least four Connecticut towns. Entomologist David E. Leonard of the Station says that this wasp is considered to be an important biological control in European woodlands. He notes that it may help to reduce the number of gypsy moths in Connecticut.

All hamburger contains blood, a natural coloring agent. Meatmen sometimes add more blood to color the fat particles and so conceal any excess fat content of the product. Lester Hankin of the Station has worked out a way to detect this deception. The Station examines samples of hamburger and many other foods for the State Department of Consumer Protection.

Neely Turner, State Entomologist, says that 6,775 acres of nursery stock in Connecticut were inspected and certified by plant protection men of the Station in 1965. The acreage and the number of nurserymen in this State has increased by about 50 per cent since 1955.

Contributors

We thank Mr. E. B. White for permission to use in FRONTIERS OF PLANT SCIENCE the quotation, "A paper that is green inside and out is on the right track."

Of the others whose writings appear in this issue, Dr. H. Paul Rasmussen is a plant physiologist on the Station staff; Dr. Pappachan E. Kolattukudy, a biochemist; and Dr. James G. Horsfall, a plant pathologist and director of the Station. Liberty Hyde Bailey, 1858-1954, was a distinguished botanist, essayist, and advocate of respect for the holy earth.

We also thank Mrs. Andrew L. Winton, Wilton, Connecticut, for the photograph of the Station staff in 1888.

New Publications

The publications 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

C202 (Revised) *Iris Borer and its Control.* John C. Schread.

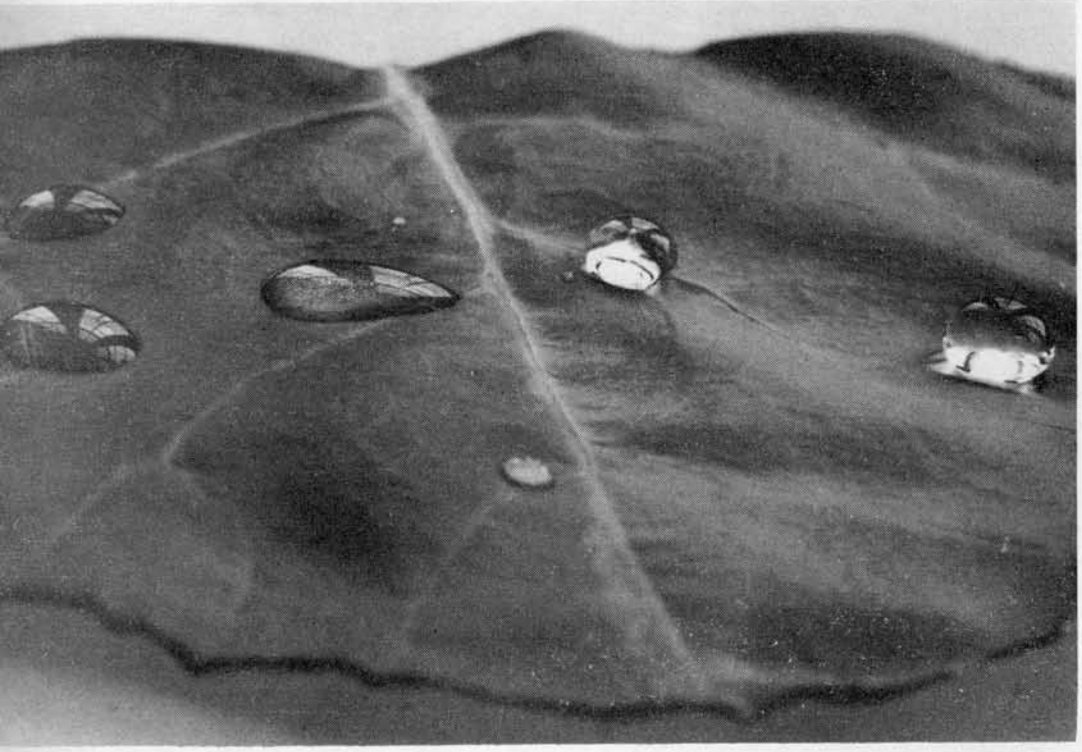
C211 (Revised) *The Black Vine Weevil.* John C. Schread.

Fungicides

B676 *Selectivity of Fungicides.* James G. Horsfall and Raymond J. Lukens.

Report on Inspections

B675 *Commercial Feeding Stuffs, 1964 Report on Inspection.* H. J. Fisher.



A natural waxy cuticle made up of some 30 compounds waterproofs the right-hand half of this broccoli leaf. When the wax is removed (left) the drops of water spread over the leaf surface.

Editor's Note

Some of the technical words Dr. Kolattukudy uses may be unfamiliar to the reader.

Chromatography is a separating of compounds by allowing a solution or mixture to seep through a blotter-like medium so that each compound or class of compounds shows up as a separate spot.

Terpenes are compounds found especially in the oils that give a plant a characteristic odor. *Aldehydes* and *ketones* are compounds chemically related to alcohol but with fewer hydrogen atoms.

Polycyclic means having several rings of atoms, and a *carbon chain* refers to carbon atoms united like a string of beads.

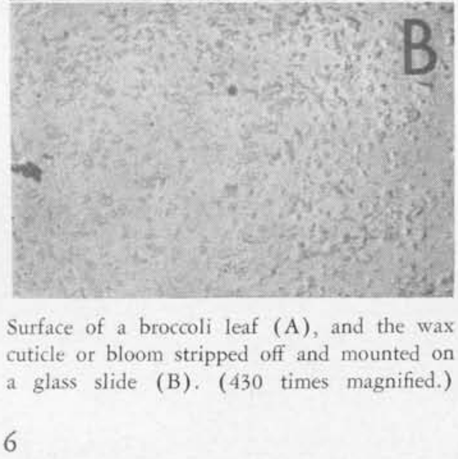
Biosynthesis is production of a chemical compound by a living organism, as the cabbage plant makes the wax covering of its leaves.

These compounds and processes are related to good eating as well as to the world of the chemist. For example, coleslaw is a mixture of compounds in cabbage leaves (terpenes, aldehydes, carbon chains, and many more) commonly also including salt, fats, oils, or acids, or all of them. Biosynthetic products, for the most part.

The Thin Wax Film of Leaves

On a Broccoli Leaf, a Mixture of About 30 Compounds

Pappachan E. Kolattukudy



Surface of a broccoli leaf (A), and the wax cuticle or bloom stripped off and mounted on a glass slide (B). (430 times magnified.)

MOST PLANTS have a water-repellant coating of fatty material, commonly called wax, on the surface of leaves, stems, and fruits. On some leaves, such as cabbage and broccoli, the surface wax shows as a "bloom" composed of white crystalline particles. On other plants the surface wax may give only oily appearance. Some plant waxes are commercially important in the manufacture of candles, wax varnishes, shoe and floor polishes, and the like. Carnauba wax for example, from leaves of a South American palm, is widely used in a variety of products.

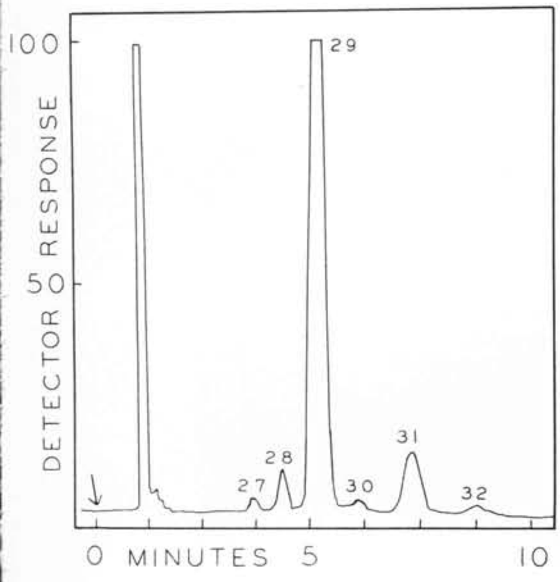
Apart from commercial value, the surface wax of plants warrants study because it is a barrier to water, carbon dioxide, disease organisms, and agricultural chemicals. Since it is the outermost coating on the plant and is chemically inert, the waxy cuticle is usually considered to be a protective covering.

Plants exchange water and carbon dioxide with the atmosphere principally through the micropores, called stomates, on the leaf surface. These stomates are opened by the metabolic machinery of the plant, thus controlling water loss. Water can be lost also from the area between stomates, which is much greater than the area of stomatal openings. If leaves had no waxy cuticle, the evaporation from leaf surfaces would be fatal to many plants. This "waterproofing" of plants is probably the most vital function of cuticular wax.

The waxy cuticle sometimes also serves as a natural defense against plant diseases. Evidence now leads us to conclude that in the withertip disease of citrus, for example, the cuticle forms an effective barrier to invading fungi. In the infection of the powdery mildew diseases, however, the cuticle seems unimportant as a barrier. Many fungi seem to be able to grow readily through the cuticle into the plant interior.

The wax keeps the surface free from moisture needed for survival of invading organisms. Furthermore, just as the wax prevents the water from sticking to a leaf, pathogens also may find it difficult to adhere. The waxy coating may also seal off nutrients or growth promotors, originating within the leaf, that are vital to the survival of the pathogen on the surface of the plant. The cuticle may also contain natural antifungal chemicals and so protect the plant from invasion.

Since the cuticle is the outermost layer, it receives full-strength the herbicides, fungicides, and insecticides applied as



A gas chromatogram of the hydrocarbon fraction of broccoli leaf wax. Each peak represents a hydrocarbon with the number of carbon atoms shown on the peak. Height of the peak indicates the amount of that hydrocarbon in the mixture. The 29-carbon peak is partly off the scale.

sprays or dusts. So this waxy covering may have much to do with the effectiveness of these chemicals. Specifically, we know that trichloroacetic acid (a relative of the acid in vinegar) reduces production of wax and thus increases the effectiveness of herbicides applied to foliage.

With the advent of new analytical methods such as thin-layer, column, and gas chromatography, the complex mixtures of compounds found in cuticular wax are readily separated. These are primarily compounds with very long unbranched carbon chains. The surface wax of an Australian cane grass (*Leptochloa digitata*) contains one of the longest carbon chains (62 carbon) found in nature. Compounds with branched chains are also found in certain plants such as tobacco. Polycyclic carbon compounds called terpenes predominate in the surface wax on fruits of apples and grapes, and on the stems of certain plants such as *Senecio*. In leaves, however, such compounds are usually found only in trace quantities.

The surface wax is chemically quite inert because it contains large proportions of hydrocarbon structures. The most common functional groups found in wax are ketones, alcohols, esters, and acids; occasionally aldehydes and unsaturation are also observed. Until very recently, little was known about how these very long chains of carbon atoms are put together by the plant. (Similar compounds are also found in insects and animals as well, even in human artery walls.)

So we set out to learn how plants build up the very long wax compounds from small molecules. Since some plant waxes are hopelessly complex, we selected a test plant which has a relatively simple mixture of compounds in its wax. The leaves of *Brassica oleracea,* which includes cabbage, broccoli, and cauliflower, were found to be especially suitable. Although the wax contains about 30 compounds, some two-thirds of it is made up of compounds with a chain-length of 29 carbon atoms.

A simple chromatographic procedure can be used to separate the wax into about seven classes of compounds. The first problem was to find out what are the basic building blocks which make up such a long chain of carbon atoms. Structurally similar chains of carbon atoms that occur in fat are known to be built up by a series of complex biochemical reactions, from 2-carbon units called acetic acid. Hence, such a building block was suspected to be involved in the biosynthesis of the long-chain wax components.

I therefore cut leaves from plants and fed them through the petiole with acetic acid labeled with radioactive carbon (C^{14}). Products formed from this radioactive carbon could then be detected by measuring their radioactivity. After allowing the C^{14}-acetic acid to react in the leaf, I extracted the wax on the surface in an organic solvent. The wax solution was radioactive, proving that the suspected building block had been converted into long-chain wax compounds. From the extent of conversion of the administered radioactivity into the wax, I determined the rate of wax synthesis under different conditions.

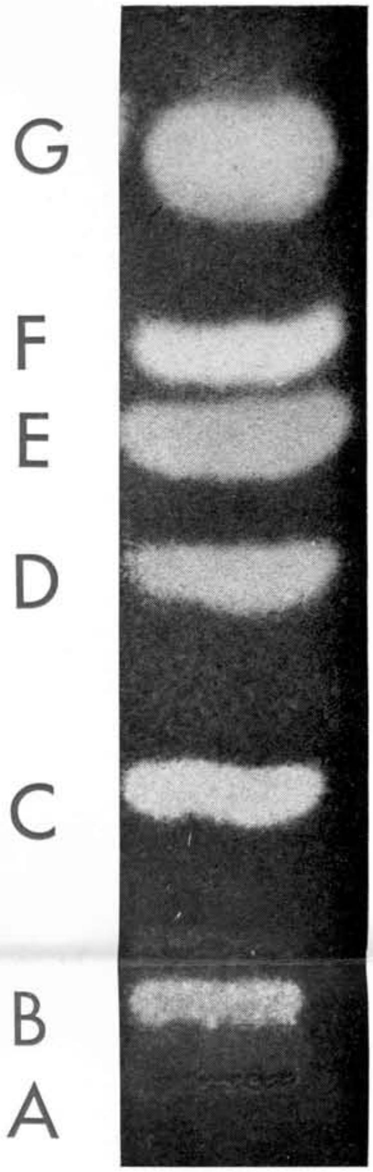
If the acetic acid was labeled by making one or the other of its carbon atoms radioactive, and if the acetic acid molecules were joined head to tail during the process of synthesis of most of the wax compounds, then the carbon chain of the wax compounds should contain the radioactivity in alternate positions. I found this to be true.

After having shown that 2-carbon units build up 29-carbon compounds, the question arises as to how this occurs in the leaf. Three possible explanations may be represented as follows:

1) 15 2-carbon units → 30-carbon compound → 29-carbon wax compound + carbon dioxide

2) 1 3-carbon unit combines with 13 2-carbon units → 29-carbon wax compound

3) 8 2-carbon units → 16-carbon compound → (loss of end carbon) 15-carbon compound
2 15-carbon compounds → 30-carbon compound → 29-carbon wax compound + carbon dioxide



Thin layer chromatogram of broccoli leaf wax showing separation into seven classes of compounds that make up the wax.

Based upon the structure of the different compounds found in the wax, the third possibility was suspected to be the most likely one.

Hence, I attempted to test the third possibility experimentally. According to this theoretical explanation, the end carbon of the 16-carbon compound is lost. Consequently the 16-carbon compound labeled only at the end carbon with radioactive carbon should not contribute its radioactivity to the 29-carbon wax compounds. It did, however, and so the third possibility could be ruled out.

The second explanation could also be ruled out because the position in which radioactive carbon was found in the wax was not in accordance with it.

Our experimental evidence so far supports the first explanation. Thus the use of radioactive isotopes gives information about the sequence of reactions that produce wax compounds.

Malthus Thwarted?

(continued from page 3)

This assumes, of course, that the world's food is distributed evenly to the world's people. Since this is patently impossible, it now seems that there will be widespread malnutrition if not famine in the seventies.

The food experts in the United Nations knew this before 1965. The man in the street and the editor in his eyrie 50 floors above discovered it in 1965.

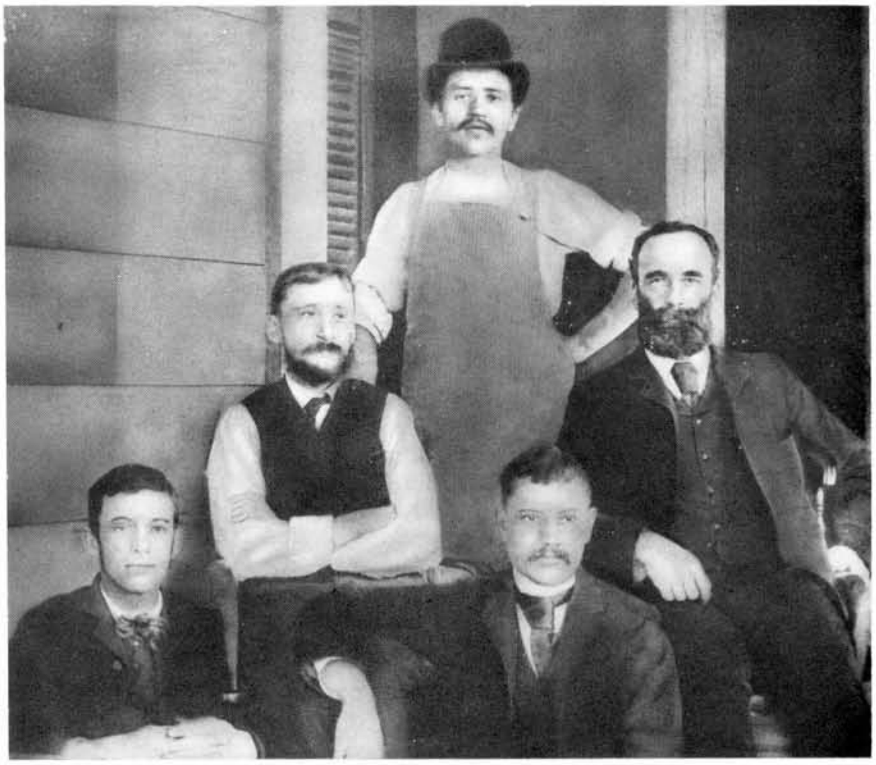
The year 1965 had its effect on our national effort. Emphasis on programs in hungry nations seems to be swinging from industrialization to "agriculturalization." As the ability of the agricultural sector to feed the people can be made to increase, the ability of the industrial sector to provide the other services will also increase.

The year had its effect at the very highest level. In late 1965 President Johnson set up a National Commission on Food and Fiber to examine our national food policies, domestic and foreign. He is asking for help from all. How can we feed ourselves and do our part to help feed the world?

And finally, the year had its effect on farmers, too. They suddenly became ten feet tall. Their position continues to brighten as the shadow cast by the surpluses over the market is removed.

Let us all pray that we and the hungry nations together can deflect the bread-people curves from their collision courses and thus continue to thwart the Malthusian gloom.

Give us this day our daily bread!



The Light of Other Days

As history is reckoned, scientific research in agriculture is a new idea. Here, for example, is the staff* of this Station in 1888, only one long lifetime ago. These men contributed to the agriculturalization (see page 3) upon which industrialized America depends. They discovered new knowledge of the land and its products. From the left, Andrew L. Winton, later chief of the Chicago laboratory, Bureau of Chemistry, United States Department of Agriculture; E. H. Farrington, later professor of dairying at the University of Wisconsin; Hugo Lange, laboratory helper; Thomas B. Osborne, biochemist on the Station staff from 1886 to 1929; and Edward H. Jenkins, appointed as a chemist in 1876, director of the Station from 1900 to 1923. The year 1888 marked a turning point in agricultural research. By action of the Congress, funds then became available to encourage every State to establish an agricultural experiment station. The potential value of these stations had already been demonstrated in Connecticut for 13 years.

---

*Not shown here, Director Samuel W. Johnson.

Frontiers
of Plant Science

Vol. 18 May 1966 No. 2

Frontiers of Plant Science
is a report on research of
The Connecticut
Agricultural Experiment Station
Published in May and November.
Available to Connecticut citizens
upon request.

Bruce B. Miner, *Editor*

THE CONNECTICUT
AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT 06504
James G. Horsfall
Director

FRONTIERS of PLANT SCIENCE
FREE • Permit No. 1136

PENALTY FOR PRIVATE USE TO AVOID
PAYMENT OF POSTAGE, $300