Frontiers of PLANT SCIENCE

SPRING ISSUE  MAY 1961

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Mountain Laurel • Photo by B. J. McFarland

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION • NEW HAVEN
As we watch another spring unfold in Connecticut, all of us must at some time reflect on the wonders involved in the growth of green leaves, a process on which our life as well depends. In recent years, biochemists at this Station have been exploring these familiar living laboratories of nature, trying to understand more about the mysteries they contain.

Sometimes we have gained insight into the biochemical reactions in plant cells by experiments outside the leaf—in test-tube fashion. Lately the tactics have varied, and it has become possible again to focus attention more precisely on processes of the intact living leaf.

We could not have predicted the results at the start of these investigations; only now do we see how our new knowledge may be used to control plant growth.

It has long been known that plants, like animals, must consume oxygen from the air in order to live and grow. Biochemists have discovered several reactions in leaves in which the uptake of oxygen occurs, and hence all of these reactions could be suspected of being involved in the respiration of the tissue. Which of these chemical reactions contribute significantly to the oxygen consumption of leaves? Under what conditions do certain respiratory reactions predominate over others? If one could specifically restrict the oxygen uptake of one of the several reactions, how would this affect the growth of the leaf? These were some of the questions raised at the outset of this investigation.

Fortunately for all of us, and especially for biochemists, the chemical reactions which occur in all living cells require the presence of large molecules called enzymes (specific proteins, each of which generally participates in only one reaction). Every cell must therefore contain thousands of different enzymes, and biochemists have devised methods for extracting and isolating individual enzymes.

One such enzyme, called glycolic oxidase, has been purified from ground-up spinach leaves until it was essentially free from all other proteins. About 15 years ago, biochemists investigating the respiration of leaves discovered this enzyme and the reaction it brings about, as is illustrated in Figure 1. In the presence of this enzyme, it was found that glycolic acid is converted to a substance known as glyoxylic acid.

The important thing to note here is that oxygen is consumed in the course of this biochemical reaction, and from this it was naturally suspected that glycolic oxidase may be involved in the oxygen consumption of leaves.

In order for glycolic acid to be oxidized, it is necessary that the protein first combine with the glycolic acid (Figure 1). The reaction of this enzyme with glycolic acid may be compared with the fitting of a lock (glycolic oxidase) and key (glycolic acid or hydroxysulfonic acid)
as shown in Figure 2. Thus any means of preventing the glycolic acid-glycolic oxidase combination would also prevent oxygen uptake.

Biochemists concerned with chemotherapy have observed that chemical compounds which are similar in structure to an essential substance may interfere with the utilization of that substance by, as it were, serving as “master keys” and “plugging up the lock.” Such studies suggested by analogy that the chemical structure of substances like hydroxysulfonic acid (Figure 2) might also “fit” glycolic oxidase and thus prevent glycolic acid from combining with the enzyme. A number of chemical compounds with the general structure shown for hydroxysulfonic acid were tested, and all were found to “confuse” the enzyme. When hydroxysulfonic acid was supplied to the enzyme isolated from spinach leaves at a concentration only one two-hundredth of that of glycolic acid, for example, it competed so successfully that the rate of oxygen taken up in the reaction was reduced by 50 percent.

If the glycolic oxidase reaction accounts for appreciable oxygen uptake by a leaf, it was reasoned that blocking this reaction might cause the glycolic acid within the tissue to increase in concentration at a rate similar to the oxygen taken up by the leaf (Figure 1).

Concentration Increased Rapidly

To test this idea, we placed detached tobacco leaves with their bases in solutions of hydroxysulfonic acid, and chemical determinations of glycolic acid were made at intervals of time after the start of the experiment. Leaves were found normally to contain a very small amount of glycolic acid, but in the presence of a suitable hydroxysulfonic acid, the concentration increased about 20-fold within 30 minutes provided the experiment was carried out in sunlight. This rapid increase in glycolic acid was similar in rate to the oxygen uptake of the same leaf.

These results meant that glycolic acid was produced primarily in sunlight, and when the further reaction by glycolic oxidase was blocked by hydroxysulfonic acid (Figure 1), glycolic acid accumulated at the rate at which it would normally have been oxidized further. Such rapid accumulations of glycolic acid under similar experimental conditions were also found to occur with leaves of tomato, spinach, bean, and corn, so that the effect of hydroxysulfonic acid is a general one in leaves.

The most important reactions of leaves in sunlight are those concerned with formation of sugars from the carbon dioxide of the air, photosynthesis. The explosive accumulation of glycolic acid in leaves in sunlight in the presence of hydroxysulfonic acid suggested that the artificial blocking of this reaction was causing much of the recently acquired carbon dioxide to be diverted from its normal products, sugars, into glycolic acid.

Determine Fate of Carbon Dioxide

To test this, we made use of the radioactive tracer known as carbon 14. Detached tobacco leaves in sunlight were placed in air containing carbon dioxide enriched with carbon 14, with the base of the leaf in either water or hydroxysulfonic acid. At the end of 15 minutes, the leaf was killed, various chemical substances were isolated from it and their radioactivity determined.

Per cent radioactivity after 15 minutes in sunlight with radioactive carbon dioxide

<table>
<thead>
<tr>
<th>Tobacco leaf in water</th>
<th>Tobacco leaf in hydroxysulfonic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars</td>
<td>63</td>
</tr>
<tr>
<td>Glycolic acid</td>
<td>57</td>
</tr>
</tbody>
</table>

In this way it was possible to determine the fate of the recently acquired carbon dioxide. As is shown in the Table, a normal leaf produced primarily sugars during photosynthesis, as would be expected, and very little of the carbon dioxide taken in was fixed in glycolic acid. With hydroxysulfonic acid present, however, these relative proportions were reversed. More than half of the carbon dioxide taken in by the leaf was now converted to glycolic acid.

These experiments with radioactive carbon dioxide provided direct evidence that glycolic acid arises from early products of photosynthesis and suggested that glycolic acid must normally be oxidized rapidly by the glycolic oxidase enzyme. This reaction must then largely account for the oxygen uptake of leaves in sunlight since so much glycolic acid accumulates when its further reaction is slowed. The reaction probably functions exclusively in sunlight because glycolic acid is rapidly produced by the leaf only under conditions suitable for photosynthesis.

Thus evidence has been obtained that glycolic acid is a keystone compound, involved in two important processes, photosynthesis and respiration in leaves.

A by-product of these studies occurred in the course of experiments conducted on bright summer days, when it was noticed that hydroxysulfonic acid treatment prevented leaves from wilting under conditions where leaves in water wilted badly. This recovery from wilting occurred rapidly. Recent experiments suggest that hydroxysulfonic acid causes the microscopic pores of leaves (stomata), through which much water is lost, to close.

These unexpected observations have led to a new line of investigation aimed towards controlling the water loss of leaves through stomatal control, a problem of the utmost importance in the study of water relations and drought.

Thus, although some of the questions initially asked have been answered, new and important ones have arisen in the course of the work. This account must therefore be considered only an installment with many more exciting episodes still to come.

New Publications

Publications listed below have been issued by the Station since you last received FRONTIERS. Address requests for single copies to Publications, Box 1106, New Haven 4.

Biochemistry (Tobacco)
B 640 Chemical Investigations of the Tobacco Plant. XI. Composition of the Green Leaf in Relation to Position on the Stalk.

Entomology
B 641 Caterpillars on Oaks
B 643 Effect of Sprays on Mite and Insect Species of the Apple Orchard Floor
C 215 Control of Leaf Miners
C 210 Scales and Mealybugs

Genetics (Tobacco)
B 636 C 2, A New Mosaic Resistant Connecticut Broadleaf Tobacco

Laws
B 612 Prevention and Control of Crabgrass in Lawns
Report on Inspection
B 657 Commercial Fertilizers, 1960

Soils and Climatology
B 654 Plastic Mulching: Principles and Benefits

Wood Products
Understanding Height Growth of Trees

Connecticut woodlands cover nearly two-thirds the area of the State. Understanding how and when trees grow can help us find ways of managing woodlands to meet our changing needs.

Stephen Collins

However we view our Connecticut woodlands, we can hardly overlook the consequences of management, on the one hand, and a hands-off policy on the other. Either way, our concern is with trees, with how they grow and when they grow. To study a forest is to study trees and their growth.

In my current study of tree growth I am particularly investigating growth in height of the small trees that may one day replace the larger trees now dominant. It is by height growth, not diameter growth, that a tree attains such dominance.

We know that without enough light these saplings may die or develop flattened tops. With too much light the lower branches may thrive and the trees will not produce the “self-pruned” stems we like to see in a productive woodland.

Light intensity, then, must be within a desirable range if the small forest tree is to grow into a sound large tree. Since the light intensity is largely controlled by the large trees in the present canopy, we can think of the forest as self-regulated. The more the canopy is disturbed, whether directly by storms or indirectly through injury, the greater the amount of light reaching the small trees beneath. A drought, a fire, defoliation by insects, a blight by fungi—all are factors which cause shifts both among species and among different sizes of the same kind of tree.

The annual cycle of growth—its beginning in the spring and end in the summer or fall—may be controlled by light. Since the lifetime growth of a tree is made up of yearly increments, the annual cycle gives us a ready handle to the problem of tree growth.

My interest in height growth of trees was renewed after observing that red maples grew longer twigs under oak stands stripped by gypsy moth larvae. Was this bonus in maple growth caused by defoliation, or was I simply seeing the favorable effects of climate associated with a particular year?

Defoliation Favored Maples

Unless I found added length on field-grown maples which were not subject to the insect influence, I could be fairly certain that defoliation, not climate, favored the maples. Maples growing in an abandoned field did not grow more during the year that woodland oaks had been defoliated. This pointed to defoliation of oak as a cause of greater maple growth.

Was nutrient in the great quantity of caterpillar frass that fell while gypsy moth larvae fed giving the maples a dose of fertilizer? Apparently not, because there was no rainfall during the elongation period to dissolve nutrients from the frass.

Before seeking an explanation for the better than usual height growth of maples under defoliated oak, I needed to establish the timing of elongation and defoliation.

Scientific periodicals and books said little about the fundamental processes of height growth in woodlands, but this is not surprising because measuring the height of large trees can be both difficult and dangerous. Information on seasonal height growth was sometimes reported for trees out of their forest setting, as for instance in nursery beds, plantations, and greenhouses. Most often seedlings or very small trees were observed.

In the forest, the environment of seedlings differs greatly from that of larger trees. Generalization based on seedling response in the open would be risky for explaining tree responses in a forest. I read that red maple seedlings grew best under experimental lighting which simulated a long day and short night. The ratio of night to day, or “photoperiod,” controlled height growth, according to one recent textbook on tree physiology. Had I accepted this generalization for trees in the forest, I would have probably pressed my study no further.

Disregarding defoliation, I had noticed that height increments of maples in fields were variable from year to year, but always exceeded growth of woodland maples. Did growth occur simultaneously in both field and forest as I might infer from the textbook?

Maples in Field Grew Longer

To answer this question measurements were taken on small maple stems during the past spring at intervals beginning after the buds burst. Starting time in both field and forest was about the same, but the maples in the field grew longer during the season than in woodland growth before the end of May, or about a month earlier than the maples in the field. This coincided with the completion of leafing by the oaks which overtopped them.

I examined a woodland which was being stripped by gypsy moth larvae. The caterpillars did not consume the less-preferred understory maple until they had eaten most of the oak foliage. The feeding started when the first tree leaves appeared. The caterpillars outstripped their food supply before...
mid-June. Their feeding, which admitted more than the usual amount of light, permitted the maple in the forest to continue growing well past the date that it would normally stop. Finally the gypsy moth larvae stripped it, too. In the defoliated forest, light intensity, not photoperiod was evidently limiting maple growth.

From this natural experiment, performed with the assistance of caterpillars, it appears that understory maples (and probably other species as well) do not realize their full height growth because they don't receive enough light. A thinning of the canopy would overcome this limiting factor for the small trees.

If, as the textbook suggests, the growth of trees in full light halts because of a photoperiod, there is little that we can do to change this in the forest. But a more optimistic guess is that a year of better growth may result when the tree has an earlier than usual start. This is the hypothesis I am testing on experimental plots where tree elongation in early spring can be observed under controlled temperature and moisture conditions.

Better control of height growth of trees in the open will interest orchardists, nurserymen, and Christmas tree growers, too.

A knowledge of when height growth occurs should also contribute to the better timing of such cultural operations as thinning, fertilizing, and spraying of trees with herbicides. For instance, sprouting is apt to be least vigorous when tree reserves are lowest. To minimize sprouting we might time our thinning right after height growth is completed. Whatever way we choose to control growth, our first aim is to understand the basic process.

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**Summer Treads on Heels of Spring**

Plan now to visit the outdoor laboratories of the Station on SCIENCE AT WORK Day, Wednesday, August 9. Tour the test plots, meet the staff, hear short talks on lawns, insects, soils, fruit growing, and foods. See how science finds answers to many of the questions we all ask. The program begins at 10 a.m. No parking problems. Follow signs on Whitney Avenue at Evergreen.

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**Lockwood Farm Mt. Carmel**
Climatologists Look Back to Make Predictions

Christopher Bingham

One of the chief concerns of the scientist is the future. The chemist experiments today to be able to predict what will happen tomorrow. The same is true of the biologist, the physicist, the psychologist, and the climatologist. All are seeking regularities in the present and past which will remain applicable in the future.

The climatologist or climate scientist is as much concerned with prediction as is the daily forecaster, and his role is no less important. There is a fundamental difference, however, between his work and that of the forecaster. The forecaster ventures forecasts for 1, 2, 5, or even very uncertainly for 30 days; the climatologist confidently predicts 1, 5, or 10 years in advance.

It is clear that this can be done only in a statistical sense. His prediction is in the form of a statement of probability. He says, for instance, that the probability of a frost after May 12 is 1 in 4 for Mt. Carmel and means, precisely, that on the average there will be one such occurrence in 4 years and 2 in 8, etc.

Using modern statistical methods, he transforms data recorded in the past into probabilities for the future. This can be limited to a simple count of the number of occurrences of an event in the past. This approach, however, can ignore important information, and hence more complex methods are necessary.

The utility of climatological forecasts is clear. A farmer can plan his planting to reduce the danger of frost while still getting his crop to market early enough to command a premium. A construction engineer needs to know the probability of high wind speeds to design a structure with the greatest economy of materials. An air-conditioning firm can use climatological forecasts to decide when to expect a sales peak due to hot weather.

An example of climatological methods is shown by our work here at the Station. As part of a regional program to develop methods making more efficient use of existing records, we studied the weekly average temperatures over a 30-year period at New Haven and Storrs, and at other locations from Maryland to Wisconsin. Our objective was ability to make accurate statements of probability about temperatures for any week in the future.

There are two important pieces of information to be gleaned from past records—the central or expected value of the temperature for each week and the distribution around this value. The distribution is expressed in terms of the probability of various departures from the central value. The most common such distribution may be shown graphically by the familiar normal or bell-shaped curve.

The first step was accomplished by fitting a smooth curve to the averages over 30 years of the weekly temperatures. We obtained an excellent fit to the observed values by a simple sine curve. (See Page 7.) Since only three numbers are needed to specify the curve, this gave us a practical method for determining the average temperature for each of the 52 weeks of the year.

Next we studied the distribution of the average weekly temperatures. Statistical tests showed that for the majority of applications the normal distribution would generally be adequate. Since the exact form of this distribution depends on its variance—the range of chance scattering around the central value—some method had to be found to estimate this. Preliminary sample estimates of the variances were made for each week, and then, for technical reasons, transformed to their logarithms. Since there was a relatively smooth transi-
tion from high variance in winter to low in summer, a sine curve was fitted to these values and then transformed back to antilogarithms. The result was a smooth curve, again determined by three numbers, giving a value for the variance for each week.

Thus given a total of six numbers for a given location, the mean and variance and hence all probability levels can be determined for any week. The Figure gives for Storrs the band in which the average weekly temperature will fall 50 per cent of the time. For example, in 5 years out of 10 the average for the week of May 15 will be between 54° and 59° F.

By such methods as this, climatologists are developing better and more accurate climatological predictions.

Area enclosed by 25 and 75 percentiles for weekly average temperatures at Storrs, Connecticut. The average weekly temperature will fall within the shaded areas 5 years in 10.

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**The Riddle of Apical Bleach**

Careful study does not always reveal a cause of plant disorder. Instead the scientist may only eliminate from consideration one logical suspect: in this study, calcium, found to be not guilty.

Dr. Waggoner had already suspected this. Consequently we applied calcium sulfate, or gypsum, to some plots on a farm where he had observed the disease. Also, in June and July just before apical bleach develops, we applied foliar sprays of calcium chloride, calcium acid phosphate, and Bordeaux mixture, which contains calcium.

Then one hot July day we counted the number of diseased leaves in all plots. We were fortunate, for the disease was widespread in the untreated plots. Of all the treatments, only Bordeaux mixture was effective. Sprays with Bordeaux mixture nearly halved the number of leaves with apical bleach.

Why had Bordeaux mixture been so effective? Was it the best form of calcium for the plants and so prevented a calcium deficiency? To answer this, we obtained some radioactive calcium for greenhouse experiments. We used this to prepare the same sprays used in the field: calcium chloride, calcium acid phosphate, and Bordeaux mixture. A very small quantity of each chemical was applied at the base of potato leaves. After 24 hours a half-inch circle of leaf where the calcium had been applied was cut out. This circle and the remainder of the leaf were each analyzed for radioactivity with a Geiger counter. If calcium had penetrated the leaf and moved out to the tip then radioactive calcium should be detectable in the leaf tips. We found that calcium applied as calcium chloride and calcium acid phosphate had entered and moved to the leaf margins while calcium in Bordeaux mixture remained in the circle where it had been applied.

If the apical bleaching resulted from a lack of calcium then calcium chloride and calcium acid phosphate should have prevented the disease while Bordeaux mixture would have been ineffective. In the field we had found just the opposite situation. Bordeaux mixture reduced the apical bleach, but not by supplying calcium to the leaf tips. Consequently we conclude that calcium is probably not involved in the apical bleach disease of potatoes.

Lloyd V. Edgington, on the staff in plant pathology since 1957, studies Dutch elm disease as well as diseases of potatoes. He is a Wisconsin Ph.D.
From the Director

A Station editor has unusual opportunities to observe scientists at work and to appreciate what they are trying to do. I have invited our editor, Bruce B. Miner, to be guest columnist in this issue of Frontiers. He writes on "The Good and the Evil."

James L. Hargrave

"It is a governing principle of nature," wrote James Fenimore Cooper, "that the agency which can produce most good, when perverted from its proper aim, is most productive of evil."

I suspect that many who view science from the sidelines share the uneasiness that prompted Cooper to call for vigilance lest "that which was established in the interest of the right, may so easily become the agent of the wrong."

Science became established because men wondered about the world around them. They sought knowledge. While science came from this desire to know, scientists have surely encouraged the belief that from science can come good. And, stated unscientifically, we know in our hearts that this is true.

Why So Uneasy?

How is it then that today, with more men and money devoted to scientific inquiry than ever before, we seem to be so uneasy, so insecure, so fearful of second place?

Can it be that we simply are unwilling to face up to what scientist Raimon Beard of our staff has so well named the "uncomfortable decisions" that inevitably accompany new knowledge?

On the one hand, we know that men of good will can use science for good, on the other that men of ill will can use it for evil. We hope that they, meaning those in authority, will make the uncomfortable decisions wisely, in full realization that science is an invention of man, a servant, not a master. For ourselves, we find it easier, perhaps, to plead ignorance. We may choose to say that science is too much for us and possibly too much for scientists, who must go on in search of truth irrespective of the use others make of their discoveries.

And so we are uneasy.

Surely there are many signs that a more comfortable outcome could attend our scientific endeavors. We know that the tools and technology derived from science can help millions free themselves from the age-old fears of famine and pestilence. In due time science may even help to batter down some of the walls of prejudice kept in such good repair by ignorance and fear.

Science and other ways of learning may be able to show that the unknowns faced by all peoples are infinitely greater and, in the end, more important than the differences that divide us.

Are scientists equal to the challenge? We can hope that they are, but a great deal more history will be written before we know.

A Thin Line

I do know that the scientists I have observed seek to stay free from bias of opinion, but they are concerned as to how their findings are used. They also know how easily the agent of the good can be perverted. After all, it is a thin line indeed that separates knowledge of plant protection (meaning food) from plant destruction (which may mean famine).

Some of these scientists may even wonder, as I do, whether we shall one day see from scientific inquiry a new quite unexpected example of serendipity. Walpole coined the word from the fairy tale of the princes of Serendip, who were always making happy discoveries by accident and sagacity, of things they did not seek.

Is it possible that by accident and sagacity, in quest of new knowledge of nature, we may one day discover better ways of getting along with each other?

If this should happen, then scientists and non-scientists alike may relax and cultivate their gardens, if not in the best of all possible worlds, at least in a better one.

Relax, that is, until some spaceship from an unseen star in an unknown galaxy comes zooming in with word that our whole world is in second place, eons behind, and we the laughing-stock of all creation.

Science is the discovery and formulation of the laws of nature. In our enthusiasm we may forget that a law not only tells you what you can do, but what you cannot do. When we use our knowledge of natural law for specific problems we are practicing technology, not science.

* * *

Modern society seems incautiously rich in means, impoverished in ends. The dazzling success of science in placing facilities at our disposal has left us all, including the scientist, a bit confused... while the scientist possesses no special magic or superior methods for reaching policy decisions, he can offer sound knowledge, highly relevant to the making of value judgments.

Paul B. Sears

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