FALL 1969

Frontiers of Plant Science

After 46 years, hardwoods take over a field and dominate this unmanaged woodland in Portland.

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION NEW HAVEN

The deer fly, about ½ inch long, bites man and many other animals.

The natural habitat of salt marsh deer flies. The object is to control the flies and still save the wetlands.

Delta-Winged Combatants of the Salt Marsh

John F. Anderson
Entomology

A SUCCESSION of insects including the mosquito, punkie, horsefly, deer fly, and even the non-biting chironomid midge (see FRONTIERS, Fall 1966) are an irritation to native and visitor alike along the shoreline wetlands of Connecticut. The deer fly is the least known insect biologically, though it is probably the most provocative.

Deer flies, mango flies, ear flies, pine flies, or whatever name one prefers, these delta-winged combatants are one of the most obnoxious pests of man and beast. These flies along with their closely allied relatives, the horseflies, belong to a large and very important group of insects called tabanids. Aside from the injury inflicted by their bite, deer flies are also important vectors of some human pathogens. A disease called loiasis is endemic to certain regions of Africa, and the deer fly is the intermediate host and vector of the causative agent, a round worm or nematode. In western United States, deer flies have been implicated as an important vector of the bacterium causing the disease known as tularemia. However, no human disease problem in Connecticut or throughout the eastern United States has been linked to the assaults of these flies.

Deer flies occur throughout the state but there are very few localities where their legions are of greater magnitude than they are in the Beaver Brook, Rivercliff, and Laurel Beach areas of Milford. Situated adjacent to the mouth of the Housatonic River and bounded by vast acreages of salt marsh, these communities have been plagued for several years by seasonal attacks of these flies. The severity of the problem is perhaps illustrated by the activities of some citizens this past year. In early March, a mother initiated a petition that was signed by over 500 residents in a single day protesting the seasonal presence of deer flies. A group of mothers, armed with this petition, then proceeded to "march" on City Hall. Numerous letters relative to the fly problem have been published in the *Milford Citizen,* and this past summer an organization has been formed whose expressed purpose is to rid the community of deer flies through "voting power."

Controlling deer flies is a formidable task for the biological scientist as well as for the political scientist. Adult flies are immune to contemporary insecticides and larvae apparently are resistant to all but the persistent chlorinated hydrocarbon ones. Filling of larval sites is effective but the wetlands are destroyed in the process. In short, there is no conventional method for managing populations of deer flies. We are interested in developing methods that might be used to suppress future outbreaks of these flies.

Deer flies in Milford belong predominantly to the species called *Chrysops atlanticus*. Two other species, *C. fuliginosus* and *C. niger*, are also present but are not as noticeable either because they are less abundant or are less rapacious. *C. niger* is the first to occur during the year, with emergence underway during May. Large numbers of the other species are not on the wing until the latter part of June and the early part of July. As with mosquitoes, only the female bites. Abundant numbers make their way to the perimeter of the marsh where man has staked out what he thought was his own private domicile. After the female feeds on man, the eggs mature, and she subsequently returns to the marsh to lay them. Within 5 to 10 days the embryo completes its development and hatches. The larva burrows into the moist but not permanently inundated soil where it remains until transformation into a pupa the following year. The pupal stage lasts for 7 to 10 days and ends when the adult emerges from its earthen environment and becomes airborne.

While considering various methods of controlling these flies, Mr. Frank Kneen, a health officer in Milford, and I decided to try to suppress populations by temporarily flooding the larval and pupal sites with "captured" perigee tidal waters. Deer flies are semi-aquatic organisms in the larval stage and can withstand inundated soil for long periods of time but our evidence indicated that the pupa could not do likewise.

In our first test in 1967, we selected a marsh where a large number of larvae were present along a brook and drainage ditches. Impoundment was effected by placing a dam at the outlet of the stream and holding the water at a height of 1 to 3 feet in areas occupied by juvenile deer flies. Water was retained in the marsh just before and during the pupal period of the flies. Our data, based on number of juvenile deer flies just before, during, and after flooding, indicated that the population had been suppressed.

The following year an experiment was designed which enabled us to flood part of a marsh and leave another in its untouched condition so that population trends of the young flies could be followed in flooded and unflooded marshes simultaneously. Deer flies were decimated in the impoundment whereas they were able to complete their development in the untreated area.

Our findings suggest that populations of deer flies can be managed by proper manipulation of water during periods of the larval-pupal and pupal-imaginal molts. The flies would be destroyed by either one or a combination of the following means: (1) larvae and/or pupae would drown; (2) larvae would be forced from beneath the surface of the soil and into the water where they would be subject to predation by fish; (3) larvae would be unable to find a suitable place to pupate and perhaps molting would be difficult or impossible; and (4) adult flies would be unable to emerge successfully from their pupal skin when submerged.

The problem in Milford has not yet been resolved. The salt marsh is extensive and much of the acreage will be difficult to impound. Furthermore, some citizens have objected to the temporary flooding procedure. This coming year, efforts will be directed towards evaluating the possibility of trapping female flies on decoy traps placed in yards. These traps will be large black lures coated with a sticky substance that will catch and hold the flies. Traps have been reported to have captured upwards of 1,000 tabanids an hour in heavily infested areas of Manitoba, Canada. A preliminary test this past summer indicated that traps placed at a height of 6 feet above the ground were attractive to *C. atlanticus*. However, this experiment did not indicate whether such traps could reduce the number of flies to non-annoyance levels.

The answer to managing deer flies probably rests in an integrated approach. That is, more than one control procedure will have to be used. Presently, impoundment of some marshes coupled with trapping may offer some relief.

Taking marsh samples with a bog saw.

How Plants Grow From a Single Cell

Regulation Is the Key

Milton Zucker

Plant Pathology and Botany

A CENTURY AGO Samuel W. Johnson, director of The Connecticut Agricultural Experiment Station, published a book entitled "How Crops Grow." His publication became a widely read classic in agricultural chemistry. To celebrate this centennial, the Experiment Station recently sponsored a series of commemorative lectures by outstanding biologists.

In the first of the lectures, Professor F. C. Steward described research which was of particular interest to my work here at the Station. Professor Steward told how he and his colleagues at Cornell University grew a complete plant, flowers and all, from a single, living cell. They obtained the cell from an ordinary garden carrot.

That cell (and probably most other living cells) had a tremendous biochemical potential. It knew how to produce all of the different kinds of tissues found in the carrot plant, from green leaf to orange root. But, had the tiny cell remained in the root and developed normally, most of its potential would have been repressed. It would have used only a small part of its inherited biochemical know-how.

I am greatly interested in the question of why cells with such great potential usually choose only a relatively few biochemical functions to perform during their lifetime. Powerful regulatory systems must operate to limit the biochemical development of cells in an organism. The study of these control mechanisms will provide new information on how crops grow.

Much has been learned in the last decade of how living organisms direct and control their chemical activities. As you might expect, most mechanisms regulating the smooth biochemical development of a growing plant or animal must be intimately interwoven. Tightly linked connections of this type are found at many levels of biological organization. For instance, ecologists speak of a "balance of nature" to describe a series of interdependent population controls. A balance of nature at the molecular level is also essential, but it makes the study of individual regulatory systems very difficult.

Fortunately some biochemical reactions can be manipulated experimentally without seriously affecting the plant as a whole. I have been studying one such set of biochemical events concerned with the production of phenolic compounds.

Living plants make an amazing variety and quantity of phenolic components. These chemicals range in structure from simple compounds that make roses red to very tough, complex lignin polymers that give wood its great strength. Lignin, next to cellulose is the most abundant organic material produced by living organisms.

The production of phenolic compounds can often be altered greatly by changes in environment. Witness the variation from year to year in autumn coloration (the scarlet colors of a New England fall are for the most part phenolic anthocyanin pigments). This environmental sensitivity suggests that regulatory mechanisms controlling phenolic production can be altered experimentally.

My studies of phenolic compounds in plants were undertaken partly in hope that some of these control mechanisms could be characterized. The work has uncovered one of the regulatory sites that plants use to control the production of phenols. This regulatory system governs the formation of a particular enzyme in plant tissues. The enzyme, called phenylalanine ammonia-lyase (PAL for short), initiates the very first reaction in a whole chain of chemical events leading to the formation of phenolic compounds. The reaction is therefore the gateway to phenolic synthesis.

Experiments with slices of potato tubers first demonstrated that a plant tissue can control phenolic production by regulating the formation of the PAL enzyme. Cells inside the potato contain almost no enzyme and very little phenolic material. But when the tuber is wounded by cutting it into slices, the cells soon begin to make the PAL enzyme. Subsequently, as a result of enzyme activity, phenolic compounds appear.

Chemical inhibitors of enzyme synthesis have been used to stop the slices from making the enzyme. Then no phenolic compounds accumulate. Oddly enough, the formation of the enzyme can be stimulated by placing the underground tuber tissue in the light. Then more phenols are produced. Actually light has long been known to stimulate phenolic production in a number of plants. The unexpected light stimulation of PAL enzyme formation in potato slices provided the key to understanding a number of the classical light effects.

Wounded potatoes use phenolic

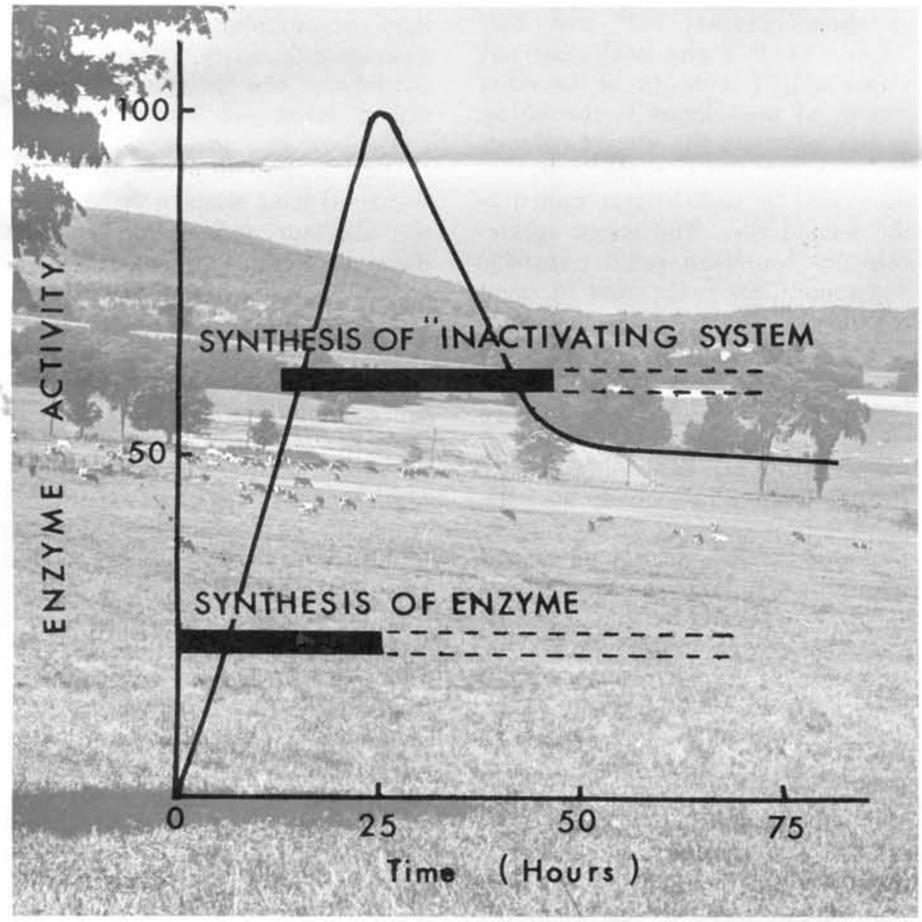
compounds to form a new skin-like material over their cut surfaces. To produce the phenols, they must turn on the biochemical machinery that makes the PAL enzyme. But what happens when enough phenolic material is made? The accumulating phenolic compounds react with the enzyme-making machinery and turn it off. Then the healing tissue begins to produce another protein capable of destroying the PAL enzyme, clearing it from the tissue. If at that point the slices are doctored with actidione, a chemical that prevents them from making more proteins, the one able to destroy the PAL enzyme is not formed. The cells are left with no way of removing the enzyme. They are tricked into keeping all they have made.

Thus the regulatory system for phenolic production in potato cells contains at least two components: one is involved in the formation of the PAL enzyme, the other in the formation of another protein capable of destroying the enzyme.

Do these studies of phenolic regulation have any potential usefulness? Yes, they offer a possible approach to increasing the nutritional value of Connecticut pasture crops. Too much phenolic production is bad for pasture plants because they use the phenols to make lignin. If you recall, lignin is a very tough material, and Connecticut cows find it hard to digest. In general, the more lignin a cow eats, the less milk it is likely to give. If the amount of PAL enzyme produced by the pasture plant could be decreased, then we might obtain a more nutritious crop with less lignin. Modification of the PAL regulatory system has been achieved with some laboratory plants. A method applicable to pasture crops could further help Connecticut farmers who have made the sale of meat and dairy products a $50 million a year industry.

Recent research from California suggests another type of application. Workers there found that a regulatory system controlling the activity of the nitrate reductase enzyme in barley also consists of two components similar to those regulating the PAL enzyme in potatoes. Nitrate reductase is a very important enzyme in agriculture. It catalyzes the first step in a series of reactions that plants must carry out to use fertilizer nitrogen for growth. A practical way of modifying the regulatory system could mean a more efficient utilization of fertilizers by crops, and in turn, more food for a hungry world.

Research on regulatory mechanisms in plants is providing a new chapter on how crops grow. That information forms the basis for future applications.



Change of PAL enzyme activity (continuous line) after potatoes are cut into slices. Bars represent the initial period of PAL enzyme formation and the subsequent period of "inactivator protein" formation.

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.

Plant Pathology

B 698 *EPIDEM, A Simulator of Plant Disease Written for a Computer.* P. E. Waggoner and J. G. Horsfall.

Biological Control

B 701 *Marigolds—A Biological Control of Meadow Nematodes in Gardens.* P. M. Miller and J. F. Ahrens.

Reports on Inspections

B 702 *The 73rd Report on Food Products 1968.* J. Gordon Hanna.

B 703 *Pesticides, Report of Inspections 1967, 1968.* J. Gordon Hanna.

B 704 *Commercial Feeding Stuffs Report for 1967.* J. Gordon Hanna.

B 705 *Commercial Fertilizers Report for 1967, 1968.* J. Gordon Hanna.

Entomology

C 231 *The Gypsy Moth in Connecticut.* Neely Turner.

Computer, Forest, and Fungus

Paul E. Waggoner
Soils and Climatology

These are the salad days of the computer. The quick mind will soon try the new gadget while common sense will ask what the new gadget actually does. Scientists at the Station have used computers to aid their study of plants, and we can now present a couple of examples to help you decide whether the computer is a genuine aid.

The first example shows the computer dealing with mountains of ecological data that would otherwise have been drudgery to analyze. The second shows the computer simulating the growth of a fungus by calculations whose complexity boggles the mind.

The troubled conservationist who worries whether deterioration of air or soil has prevented the normal growth of natural vegetation is frustrated by a lack of ecological data taken over a long period.

The data are lacking because forests change slowly and generations of scientists must keep the flame of interest alive and return to the same forest.

The data also lie in dusty cabinets because mountains of measurements must be analyzed. In Connecticut, however, the steadiness of the Station foresters has brought them back three times to ecological plots established in 1927, and the computer now speeds the analysis. In 1927 Station foresters enumerated the stems thicker than a half inch on transects through central Connecticut forests. These transects encompassed about 10 acres of land in their path over hill and dale, ledge and swamp. In 1937, 1957, and 1967 Station foresters, notably Henry Hicock, Alex Olson, and George Stephens, returned to the plots, tracing the birth, growth, or death of about 30,000 stems.

After the survey in 1957, the data were punched into cards. Tabulations showed the decline in the number of stems, the growth in the quantity of wood, and the continuing dominance of the oaks that were favorite food for the pesky gypsy moth. These were the facts that the conservationist was looking for, but as a dry history they made very dull reading. We had not yet learned to exploit the power of the computer.

In 1967, with years of birth, growth, or death of 30,000 trees compactly written on a single magnetic tape that could be examined in a few minutes, our imaginations were freed of the incubus of drudgery. We divided the forest transects into small plots and asked what transitions of forest types had occurred on them between 1927 and 1937 (Table 1). If a plot held more oak stems in 1927 than any of the other groups of trees listed in the Table, it was tallied in the second column. If it contained more oak than any other sort in 1937, it was tallied in the second row. The minor species category contained small trees like dogwood. Clearly, the most frequent or probable thing was for a plot to retain its classification, but some changes are evident. The probabilities for the 1957-67 decade look surprisingly like those of Table 1, suggesting that the transitions are steady. If they are steady and depend only upon what is growing on the plot at the beginning of the decade, then we can by algebra anticipate the steady state that the forest is tending toward.

In Figure 1 the height of the blocks shows, from the bottom up, the proportion of the land dominated at the eventual steady state by maple, oak, birch, and other major and minor species. The steady state forest anticipated from the first decade of change is on the left side of the column and the one from the last decade of change is on the right side. The similarity of the expectations from the two decades of change separated by a score of years indicates that the extrapolation is realistic. The tendency is away from the domination by oak. Thus the ease of summarizing and handling data by computer has brought us to a compact summary of the transitions outdoors in the forest and a prediction of what will happen outdoors.

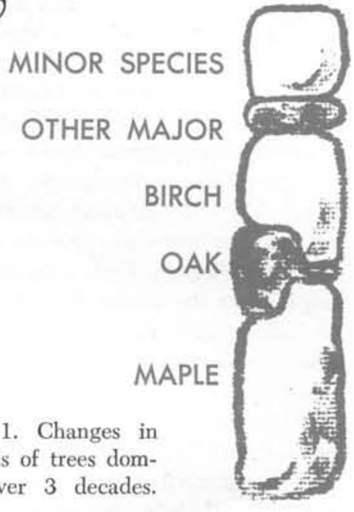


Figure 1. Changes in the kinds of trees dominant over 3 decades.

The next example of computerized ecology tests whether so-called fundamental data about a fungus from the laboratory indoors can be a fundament for anticipating the effects of weather upon the fungus as it causes a disease outdoors.

The fungus *Alternaria solani* defoliates tomatoes and potatoes. Station pathologists had long observed the fungus in the laboratory and the disease at the Lockwood Farm in Mt. Carmel. The question was whether combining the laboratory observations logically could explain the effects of weather upon the disease in the field.

The effects of an environmental factor or two upon a single fungal stage, such as spore germination, can be observed in the laboratory. But when all the facts about weather factors and fungal stages are brought together, the pathologist has had either to decide that only one factor and only one stage were overwhelmingly important, or keep all results in front of him and blow his mind with the complexity of it all. Now, however, the computer by repetitiously

Table 1. Ten-year transitions in classes of species according to number of stems per plot. "Other" means the most numerous are major species other than maple, oak, and birch. Probabilities are shown as percentages.

	1927				
Observed 1937 ↓	*Maple*	*Oak*	*Birch*	*Other*	*Minor*
Maple	82	16	13	7	7
Oak	7	72	2	3	7
Birch	2	8	83	7	7
Other	0	0	2	69	7
Minor	9	4	0	14	72

taking tiny steps can employ all the complexity from the laboratory according to simple rules.

The fungal simulator is called EPIDEM and considers the effect of weather upon the microscopic stalks and spores on infected hosts, germinated and ungerminated spores on new hosts and invisible incubating infections. We wrote rules for calculating the number of spores from the weather that embodied the laboratory observations of how temperature and so forth affected sporulation. We also wrote rules about all other stages. Then the stages were brought into communication, as when spore formation considers the number of stalks available for spores. Thus, when EPIDEM updates the accounts eight times a day, it considers both the effects of weather upon each stage and the interactions among the stages.

You may want to know how one account is handled. A flow diagram, Figure 2, shows how the number of germinated spores is calculated. At the top of the diagram we enter, knowing the number of ungerminated spores lying on leaves. First, are the leaves wet? If so, we follow the right path to the statement "germinate all." We know that 3 hours of dew promptly germinates all spores. This simple calculation finishes accounting for germination. Now, let's go back to the first diamond. If the leaves were dry, the next question concerns whether the leaves were dry 3 hours ago. This must be asked because drying kills spores that germinated in water. Therefore, if the leaves were wet 3 hours ago all germinated spores are killed. Next we calculate the number of germinated spores as a function of relative humidity, because pathologists know that some spores will germinate in humid air. Now we go to calculations for the next account.

Simple Figure 2 shows how the weather observations of leaf wetness and air humidity are employed. Although simple, it logically uses all the fundamental information in predicting how an epidemic will grow according to the weather.

Testing the Simulator

Composing the simulator is fun, searching the literature of pathology for the parts is fun, and even better is performing the laboratory experiments that have been overlooked. But sooner or later, the moment of truth arrives. Is the simulator realistic? This is tested twice. Since the simulator is a mirror of nature, the first test is asking whether the parts, such as spore germination are realistic.

The second test is feeding real weather data into the simulator and finding whether EPIDEM recreates the epidemics. Weather and epidemics had been observed at Mt. Carmel, and 1941, 1943, and 1944 had different amounts of disease. Happily, EPIDEM mimicked the differences in disease amongst the years. Epidemics in another variety of tomatoes in 1950 and 1951 could also be compared, and EPIDEM mirrored the differences.

The simulator needs more testing, but in the meantime we can accept its realism and use it for synthetic experiments. For example, what would happen if the leaves were wet with dew for 14 hours a night and then dried by a hot sun as in Israel? We asked because surprising epidemics have appeared in that dry place. EPIDEM told us that the alternation of dew and hot winds would germinate the spores during the night and spread the spores during the day, producing more disease than ever observed in Connecticut.

I began my article by saying that the quick mind might take up computers, but common sense would ask that they be useful. Now the reader has seen these gadgets at work. In the first example the 40-year histories of 30,000 trees were reduced to a compact table of transitions of trees and an extrapolation to a steady forest of the future. This study is more fully described in Station Bulletin 707, now in press. In the second example the facility of the computer for many small steps was exploited to use fundamental laboratory observations of a fungus for anticipating its growth outdoors. We reasoned simply and then by communications set up within the simulator considered interactions as well as the diverse affects of several weather factors upon different stages in the fungal life. This investigation is more fully described in Station Bulletin 698.

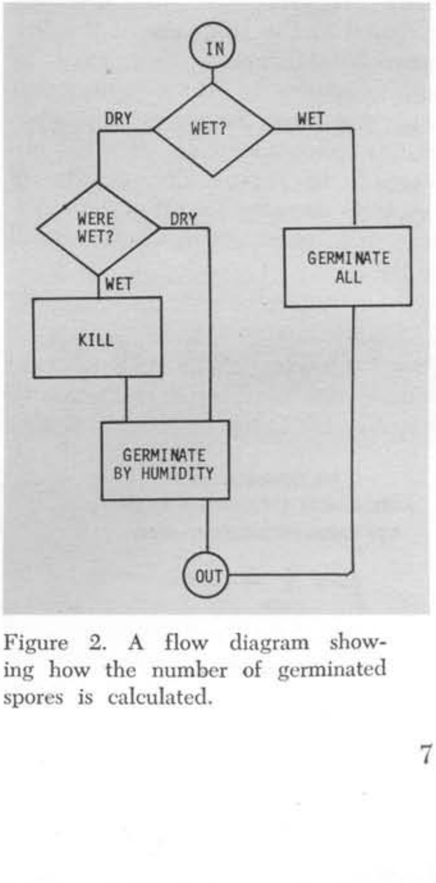


Figure 2. A flow diagram showing how the number of germinated spores is calculated.

One View of a Citizens' Experiment Station

Bruce B. Miner

Editor

As the sixties end, it may be appropriate to take a candid look at the primary responsibilities of this oldest American Experiment Station as they have evolved over 95 years, and see how they are met.

The responsibilities arise from the unique relation of the Station to the State.

As a Connecticut citizen, you have in this Station a group of professional scientists engaged to conduct original research in agriculture and "kindred interests" for the people of Connecticut. Other states employ scientists in these fields. What is unique here in Connecticut is that the Station, not being part of a university, is directly responsible to your elected representatives.

Not beholden to an administrative hierarchy, nor buffeted by irate alumni, the Station addresses itself to two primary responsibilities (without overlooking important statutory duties).

The first responsibility of the Station—its reason for being—is to conduct original scientific research and publish the results. Since these primary reports are of necessity couched in the language of the Science Establishment, they may be unintelligible to those unfamiliar with that lingo. So a second responsibility follows logically. It is the obligation to report the results of research directly to interested citizens of Connecticut in a form useful to them.

How well does the Station meet the responsibility for scientific research? As you may know, scientists themselves constantly evaluate the work of their contemporaries, largely through professional societies and their publications. We laymen are free to take their findings on faith, or form our own opinions. By their peers' evaluation of professional eminence and competence, world-wide, this Station ranks high, as I read the record. An impressive list of honors to the staff awarded by diverse groups suggests that many non-scientists concur, in Connecticut and beyond.

Reporting the results of research to citizens in a form useful to them —the second responsibility—is a more nebulous thing. During the sixties the Station has published 97 bulletins on its investigations. The information in these and in some 800 technical papers published in journals during the decade is a large part of our current stock in trade. We make this information available to Connecticut citizens in various ways.

We have distributed approximately 100,000 copies of Frontiers of Plant Science, provided science stories and additional material to the news media and correspondents, and made hundreds of talks to growers, gardeners, and others with widely varying interests.

Our staff has regularly provided information for writers, for planners, for health officials, and for legislators. We have answered thousands of questions for citizens.

We try to make clear that results of research are not recommendations but rather evidence to be considered when choices are made.

We hope people understand that a report of research is the latest word, not the last word. We have no gift of prophecy. No one can yet foresee all of the consequences of manipulating elements in our environment. As Dr. Waggoner points out in this issue, the computer vastly improves scientific estimates of probable interactions. But the mindless computer remains a tool, not a scientific Ouija board.

Having done what we can to inform the citizenry, taking pains to be honest and believable, we hope for the best.

How well the Station meets this responsibility is for Connecticut citizens to say.

How serviceable the Station will be in the seventies depends upon the quality of research, its relevance to the problems then at hand, and diligence and imagination in putting it to work for the people of Connecticut.

With some 600 people to the square mile as the decade begins, and more to come, Connecticut will face no dearth of problems in the seventies.

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