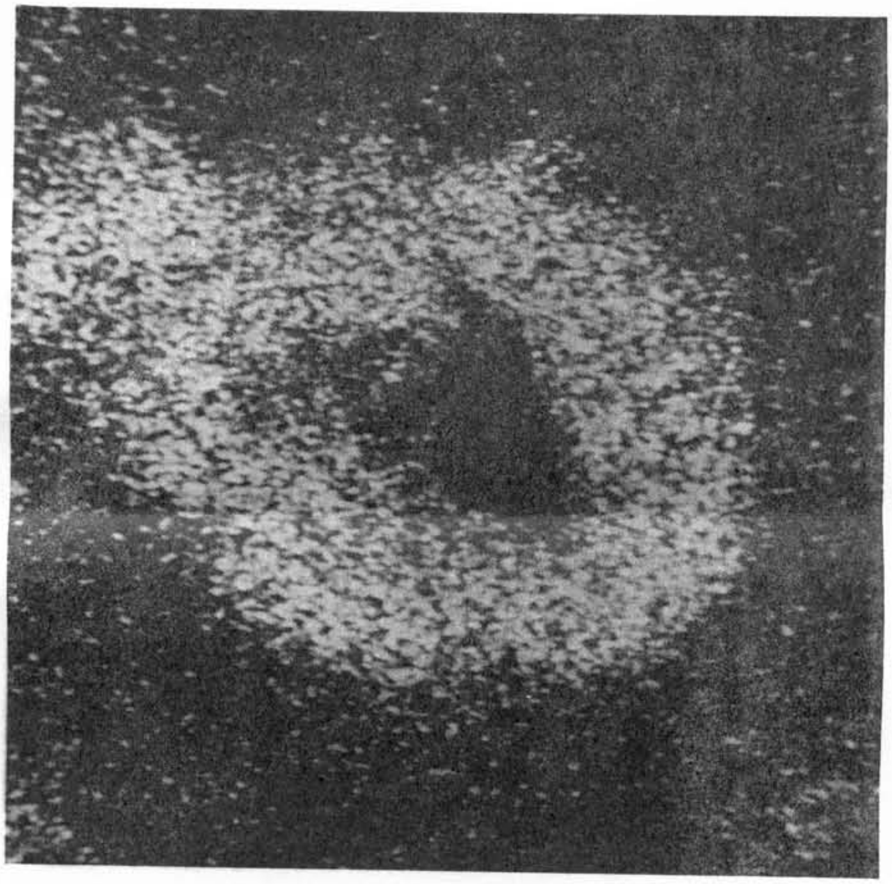
SPRING 1971

Frontiers of Plant Science



Potassium accumulation in guard cells of leaf stomate
. . . see Stomata and the Potassium Pump, page 2

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION NEW HAVEN

Stomata and the Potassium Pump in Plants

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STOMATA, the tiny pores on the surface of plant leaves, permit flow of carbon dioxide, oxygen, and water vapor for vital processes of plant growth—photosynthesis and respiration. Thus, if these processes could be regulated through artificial control of the stomatal aperture, savings in water consumption and increases in food production could result. For successful control of the stomatal aperture, however, an understanding of the mechanism of opening and closing of stomatal pores is essential.

We know that stomata in most plant species open in the light when the guard cells on either side of the stomata take up water and become more turgid than the adjacent cells on the leaf surface. Opening of stomata thus requires the movement of water into the guard cells and the adjacent cells. Sugars produced by photosynthesis in guard cells have long been thought responsible for water movement.

Recent investigations, however, suggested that accumulation of potassium in the guard cells is necessary for water movement, and hence for stomatal opening. We have tested this hypothesis by using the electron microprobe technique which permitted quantitative determination of potassium concentrations in regions smaller than one-millionth of an inch within individual guard cells. For qualitative estimates we obtained pictures of the potassium distribution in larger areas containing a pair of guard cells and adjacent cells. Our results show that stomatal opening is controlled by active transport of potassium into the guard cells.

We used tobacco leaves from greenhouse-grown plants and placed them in the dark for several hours so that the stomata closed. From these plants, leaf discs were cut, placed on water and illuminated to obtain open stomata. Then the discs were placed in the dark for varying periods to obtain different stomatal apertures. After these treatments, portions of the lower surface of leaves were quickly stripped off and freeze-dried under vacuum. The freeze-dried samples were then analyzed by the electron microprobe.

The photographs on the left show the portion of the leaf analyzed and the photographs on the right show the distribution of potassium in the corresponding area. The number and brightness of the white spots is proportional to the concentration of potassium. It is clear that the guard cells of the closed stomate (top) contain less potassium than the guard cells of the open stomate (bottom). The quantitative measurements of potassium concentrations showed that potassium in guard cells increased linearly as the stomatal aperture increased and the guard cells of a fully-opened stomate contained more than two and one-half times as much potassium as that of a closed stomate. The potassium concentration in guard cells of open

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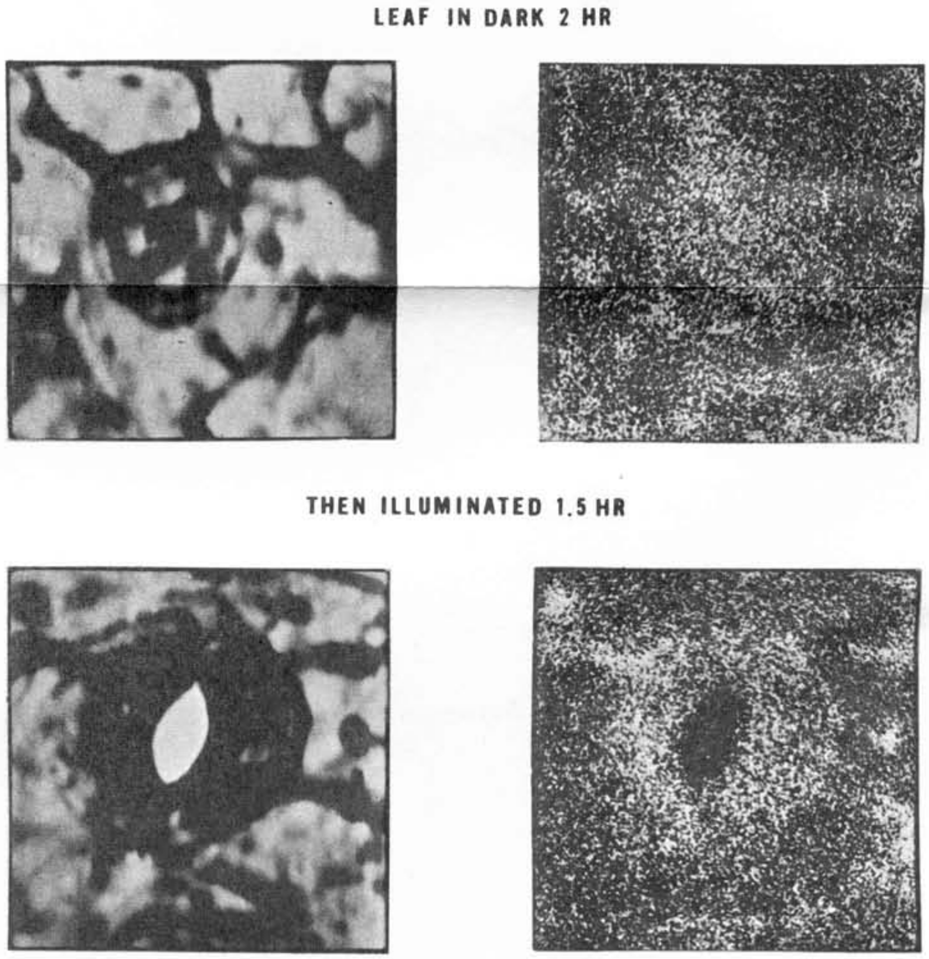

Stomata of tobacco leaves (left) and distribution of potassium (right) in guard cells of closed (top) and open (bottom) stomata. The greater number and brightness of white spots demonstrate that potassium accumulates in the guard cells of the illuminated open stomata.

50th Year for Valley Laboratory

Gordon S. Taylor
Assistant in Charge

OUR VALLEY LABORATORY at Windsor marks its fiftieth year in 1971. Starting out as a facility to solve the problems of wildfire disease on tobacco, it has since been the means for productive research in many areas of Connecticut agriculture.

Problems faced by growers of tobacco, potatoes, nursery stock, and vegetables have always received attention by the Station. The New Haven laboratory of the Station has cooperated in research on fertilizers, insect control, disease control, and crop improvement through genetics.

As early as 1892 a small experimental farm in Poquonock was established by a grower cooperative called the Connecticut Tobacco Experiment Co. Work there established the basic fertilizer practices still in use and invented the shade tent as a means of producing highest quality cigar wrappers. The company ceased supporting the research in 1905.

In the late teens, epidemics of the bacterial disease "wildfire" threatened to end tobacco production. Whereupon, the growers formed the Connecticut Valley Tobacco Improvement Association (CVTIA) to help set up a laboratory in the tobacco growing area. Windsor was chosen as suitable for all types of tobacco being grown and Messrs. F. W. Morgan, J. E. Ransom, and J. B. Stewart purchased there a farm of 13.5 acres with three curing sheds. Station Director E. H. Jenkins then reimbursed these gentlemen from the Lockwood Trust Fund.

On May 5, 1921, the General Assembly appropriated $10,000 as operating funds for the biennium with an equal amount to come from the growers. The appropriations act wisely allowed any research "for improving the crop or its preparation for market."

Dr. G. H. Chapman was chosen as Research Director of the "Tobacco Experiment Station" under a joint operation agreement by The Connecticut Agricultural Experiment Station and the CVTIA. In 2 years the wildfire problem came under control, growers ceased active management of the station, Dr. Chapman resigned, and the name was changed to "Tobacco Substation of The Connecticut Agricultural Experiment Station." C. M. Slagg and Dr. N. T. Nelson each directed the research for a year until April, 1925 when Dr. Paul J. Anderson was appointed Research Director. Facilities then included a woodframe laboratory with two offices, two laboratories and a sorting shop for evaluating tobacco, a second-hand greenhouse, and a new curing shed.

In 1941 the research staff moved into the present three-story brick building with laboratories, offices, library-conference room, and an auditorium for public meetings. A Superintendent's residence and garage were also new that year.

Two greenhouses and a headhouse were added in 1958. More land for research plots and 28 acres of adjoining woodland were acquired in 1946 and 1959, respectively. The name was changed again to Tobacco Laboratory in 1947.

For 28 years Dr. Anderson and his staff including at times, T. R. Swanback, O. E. Street, S. B. LeCompte, A. B. Pack, and E. L. Petersen learned and published about disease and nematode control, fertilizers and quality, irrigation, variety improvement, insect control, and curing. Much of the research was in cooperation with other departments at New Haven, and during these years the land at Windsor was also used for vegetable, corn, and potato research by New Haven based staff.

When Dr. Anderson retired in 1953, the Tobacco Laboratory was changed from a separate department to an arm of the Director's Office with the staff as members of various departments, but stationed at Windsor. Director Emeritus W. L. Slate took charge until July 1, 1953 when Dr. Gordon S. Taylor was appointed to head the laboratory. Soil scientist Dr. Henry C. De Roo also came in 1953 and Dr. John Ahrens joined us in 1957 as plant physiologist. The change in organization brought all of the Station's scientific expertise to bear on the many problems of

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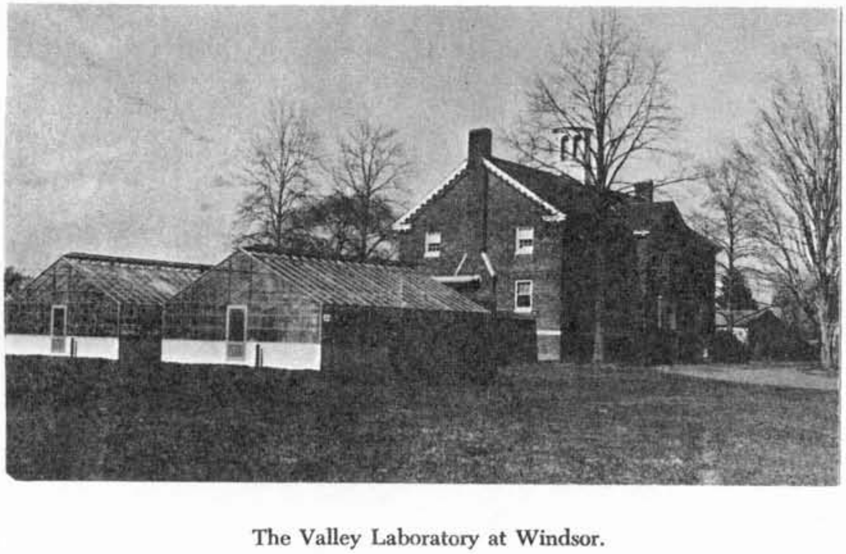


The Valley Laboratory at Windsor.

How They Differ

Infectious and Saprophytic Bacteria

David C. Sands

Department of Plant Pathology and Botany

WHY DO CERTAIN MICROBES cause disease whereas the vast majority of them do not? This primary question of plant and animal pathology has been pursued ever since Pasteur proposed the Gem Theory of Disease. If we knew how bacteria that cause disease differ from those that always live as saprophytes (on dead material), we could use this information to control disease, and perhaps improve our methods of control.

Some bacteria live in soil and water. Others cause disease in plants and yet others infect animals and man. In the laboratory we have compared the characteristics of saprophytic bacteria with forms that infect plants, concentrating on members of the group called *Pseudomonas*. This genus contains both saprophytes that live in soil and water, and a number of important plant pathogens. Bacteria can use some organic compounds for food, but whether one compound or another can be used depends upon the specific enzymes that the bacterium produces.

Our methods of comparison involve several hundred standard biochemical and nutritional tests on each of several hundred pathogens. Although such a large number of comparisons, 60,000 in all, requires computer analysis, it is obvious even without this that pathogens have important characteristics that differ from those of saprophytes. First, the pathogens are less versatile than the saprophytes. They are far more particular about their diet. Second, these pathogens grow more slowly than saprophytes. Third, many of the pathogens produce substances that are toxic to plants. We will attempt to explain how and why all of these differences occur.

First, we consider the matter of feeding habits. In the process of evolution, bacteria that infect plants or animals surely developed from more primitive types that were saprophytic. The process of development involves genetic changes and natural selection under conditions of a changing environment. When conditions favor growth, the bacteria divide every 30 minutes or so. In dividing, each cell receives some 10,000 genes, which store coded information about how to make as many different proteins. Most of these proteins are enzymes, about 1,000 of which are involved in digesting available food and converting it to the energy necessary for growth, repair, and reproduction of bacterial cells. Each enzyme is concerned with a specific chemical reaction. Thus, the number of enzymes a bacterium can produce determines how many kinds of compounds in the environment it can use for food and as a source of energy. The greater the number of compounds in soil or water that a bacterium can use, the more versatile it is, and versatility is important in the survival of bacteria that dwell in soil and water.

If versatility is important for survival of saprophytic bacteria, so is their efficiency. Every time the bacterium divides, it must duplicate each and every gene, a process that consumes energy. When a greater variety of genes is produced than is needed, too much energy and time are required to duplicate them. Such a bacterium is inefficient and does not compete well with others. Generally speaking, bacteria have maintained a favorable balance between number of genes that they reproduce and rate of growth. Bacteria improve their efficiency in another manner. Often they do not make an enzyme unless they need it. They make lactose-degrading enzymes, for example, only in the presence of lactose. At any one time they may be using only 30% of the 10,000 genes they carry.

Genes are altered at a slow but steady rate by mutation. Unless a gene is necessary for survival of a bacterium, it can be modified by mutation or lost altogether, with no harm done. This loss of genes has been demonstrated many times by placing a bacterium in solitary confinement of a test tube on rich nutrients. In time, genes are lost that are not used in the presence of a simple, unvarying diet. In this environment, the mutant survives and is efficient, it has to make fewer enzymes. But it has lost versatility as it becomes more efficient, and when put back into a natural environment in the soil, it does not survive.

We suggest that plant pathogens evolved from saprophytes. Plant materials, as they died, fell to the soil and were used as food by saprophytes. Some saprophytes on plant surfaces, adapted to conditions there. A potato tuber, a head of lettuce, or head of cabbage or celery are each fairly constant in composition. Bacteria that constantly associate with any one of these can simplify their nutritional requirements, can increase efficiency, but lose versatility by producing only the enzymes necessary to attack these organs. Such tissues are commonly rotted by bacteria, as every housewife knows.

This specialization of pathogens for growth on living tissue and usually on a particular kind of tissue seems generally to be accompanied by a decrease in rate of growth, an increase in the time required for cells to multiply. This is exhibited on laboratory media, when pathogenic forms are compared in growth rate with saprophytic ones. Saprophytic forms, obviously, cannot grow at all on living plant tissue.

The growth rate of pathogens is slow on plant tissue for another reason. In contrast with dead plant tissue, rapidly growing tissue produces compounds that are toxic to microorganisms when it is injured or invaded. These compounds are produced rapidly and are highly toxic to microorganisms that do not invade these tissues. These cannot grow on the tissue, but pathogenic forms on this tissue have developed some resistance to the toxic substances and their growth rate is merely slowed up.

When a bacterium develops resistance to toxic substances in plant tissue, it can invade tissue while it is still alive. This offers an advantage over the saprophyte, which cannot invade. Resistance to toxic substances in the plant is due to mutation but often at some cost. Two examples involve a synthetic substance called fluorophenylalanine, which is toxic because it interferes with utilization by cells of the essential amino acid, phenylalanine. Mutants of bacteria can become resistant by losing ability to let all such compounds into the cell or they can make a high amount of phenylalanine to overcome toxicity of fluorophenylalanine. One mutant loses versatility and the other loses efficiency. Each is less capable of functioning as a saprophyte but enjoys a competitive advantage over saprophytes.

The pathogenic species of bacteria, in our broad comparison, differ from saprophytes in that they frequently produce compounds that are toxic to plant tissue. If a saprophyte cannot invade host tissue, then, as evolution of a saprophyte to a parasite occurs, it may through mutation, develop a method of killing host tissue nearby its own cells, and so be able to invade the tissue as it dies.

At the outset, we mentioned the possibility of new methods of control as a product of this study. Our work is at its beginning, but we have ready methods for inducing genetic changes in bacteria. We are seeking to make pathogenic bacteria more specialized, by reducing the number of plants they can attack. Already we can report progress in changing a pathogenic bacterium so that it attacks only certain weeds. Hopefully, one outcome of this work will be a biological method of controlling weeds.

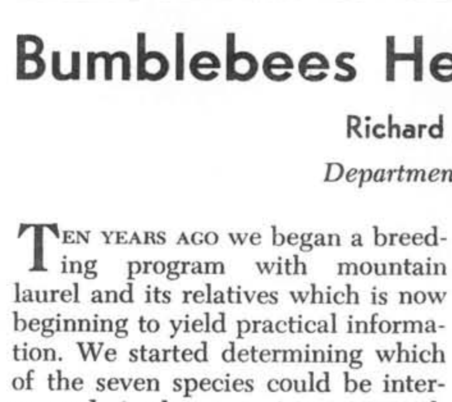
Bumblebees Help Plant Breeder

Richard A. Jaynes

Department of Genetics

TEN YEARS AGO we began a breeding program with mountain laurel and its relatives which is now beginning to yield practical information. We started determining which of the seven species could be intercrossed. At the same time, cross pollinations were made among plants with unusual or outstanding flowers or foliage within several of the species to determine the inheritance of some of these traits.

Progress is slow with these shrubs which may take as long as six years



Cage used to confine bees.

to flower from seed. We have learned, however, that certain selections of mountain laurel and sheep laurel can be bred true from seed. Red-budded mountain laurel is one of these; outstanding forms can be reproduced from seed by crossing a red-bud by a red-bud. White flowering selections appear to be reproduced in a similar fashion. Results with some of the deeply pigmented pinks are not yet far enough along to know if they can also be bred true to type.

Of course, hand pollinations are all very well for a scientist if he only needs enough seed for a few dozen plants from a cross, but what about the nurseryman who may want a large quantity of seeds to plant? We have learned that the common bumblebee will make the pollinations for us. The bees are put to work by first placing a screened cage over the two plants to be crossed before the flowers open (see Figure). When the flowers are open on both plants one or two bees, gently washed to remove foreign pollens, are introduced into the cage and they make the cross pollinations. Fortunately, self fertilization does not normally occur if the flowers are cross pollinated as well as self pollinated.

The mountain laurel, the state flower of Connecticut, is widely used in landscaping our gardens and parks. Over the last 100 years selections with great horticultural merit have appeared but have never been widely distributed to the public because of the difficulty in propagating by cuttings. By learning how to reproduce the best laurel forms from seed an alternative means of propagation becomes available. Through such studies a greater diversity of hardy plant material will become available to Connecticut citizens.

How Plants and Soil Muffle Noise

Donald E. Aylor

Department of Ecology and Climatology

VEGETATION has often been suggested for reducing community noise levels, but opinions about the value of plants for this purpose vary widely. Opinions differ partly because few data are available. Moreover, the existing data, although representing a wide range of vegetation, do not agree on how much plants can reduce different frequencies of sound. For example, two investigators found that vegetation was more effective in reducing the higher, more piercing and annoying frequencies than the lower or deeper ones. A third study found, however, that all plants reduced all frequencies about the same.

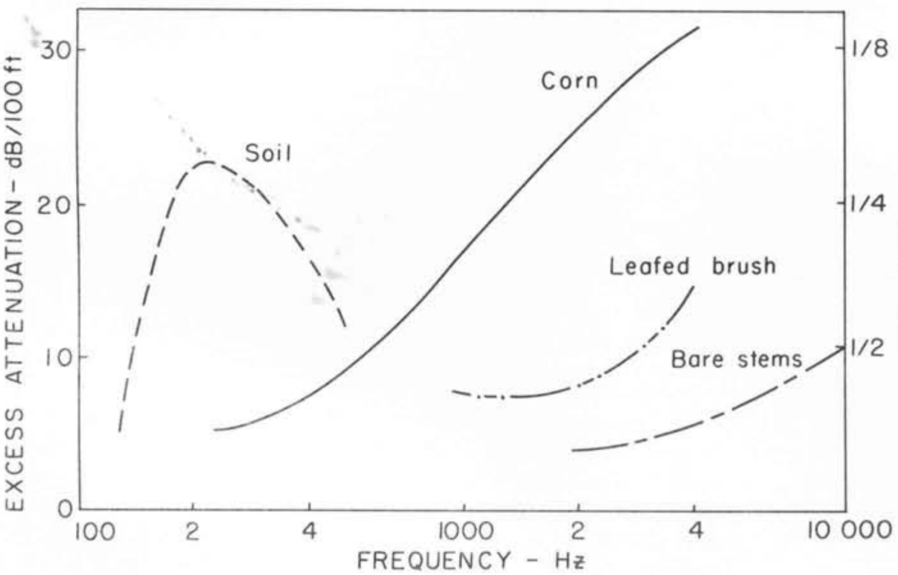
Our study of sound transmission through vegetation sought to answer two main questions. First, what frequencies of sound will be reduced by any existing stand of vegetation; and second, what type of configuration of plants would best muffle given sound frequencies? To answer these questions, some knowledge of the physics of the interactions between sound and vegetation is necessary.

To help us understand the physical processes involved, we tested vegetation with vastly different foliage area, stem diameters and densities, and ground conditions. The differences in sound transmission through these diverse sites plus some well-known theories from acoustics allowed us logically to relate attenuation to sound frequency, foliage, stems, and soil.

For this purpose we measured the transmission of sound through a dense corn crop, dense hardwood brush, a dense hemlock plantation, a thinned pine stand, and over cultivated ground. The plants on any one site were of uniform age, and all populations were of one species except the brush. The corn provided abundant foliage near the ground; its foliage could be successively thinned to measure the effect of varying amounts of leaf area on sound energy. As another measure of the importance of leaf area, sound was transmitted through the canopy of some deciduous species both in summer and again in autumn after the leaves had fallen. The leafless brush, the hemlock and the pine offered a comparison of transmission loss due to vastly different stem sizes and numbers per unit of land area. The full range of sites also provided a wide range of ground conditions. Further, we observed sound over a bare soil that had been crusted by rain both before and after tilling the surface.

The energy of sound radiated outdoors is reduced by several factors. First, sound is reduced by simply spreading out through an ever-increasing space as it travels out from its source. Moreover, the amount of sound reaching a distant point is also effected by absorption in the atmosphere and by wind and temperature profiles above the earth's surface. When the measured transmission has been corrected for these factors, the remainder represents the attenuation due to the vegetation and ground along the path, and this we call excess attenuation, Ae.

The Figure summarizes the Ae per 100 ft. of transmission path in each of the test sites. Since a 10 dB (decibel) reduction in sound energy corresponds roughly to a halving of loudness, we can equally well consider the results in terms of decrease in loudness for each sound frequency. Three clear patterns emerge from the data. Leaves absorb little sound at low frequencies but



Attenuation of sound due to various kinds of vegetation, and soil.

considerable amounts at higher frequencies. This is obvious from the corn data and is also apparent by comparing measurements made in the brush in summer and autumn.

When little foliage is present, as in the pine, hemlock and bare brush, sound is reduced either by stems or by ground. Stems are ineffective at low frequency and only moderately effective at high frequency. It is interesting that the stems of all stands tested reduced high frequency sound by nearly the same amount and thus, can be represented by a single curve in the Figure even though each stand was physically very different from the other. Soft ground, also shown in the Figure, absorbs considerable amounts of moderately low frequency sound but is ineffective at very low or at very high frequencies.

Some principles from acoustics allowed us to understand our data in a framework that is more easily generalized to any situation of vegetation and ground. Leaves and stems reduce transmitted sound mainly by scattering, while ground absorbs sound. As noticed in the Figure, soil exhibits a peak absorption at 200Hz (Hertz, or cycles per second) and reduced absorption at other frequencies. Such absorption peaks are due to resonance; the center frequency, the size and the shape of this peak all depend mainly on the permeability to air of the soil surface. In the Figure we have represented a soft, pervious soil. On the other hand, a hard, impervious soil would absorb much less and would peak at a higher frequency.

One of the conclusions of this study is that ground absorption is nonlinear with distance, i.e., it has a reduced effectiveness as distance from the source increases. This tells us that measurement made far from the sound source and standardized to shorter distances by assuming a linear reduction with distance underestimates the value of a narrow band of ground. For example, 70% of the Ae due to ground attained at 160 ft. has already been reached at 80 ft. from the source.

The loss of high frequencies due to scattering by stems is nearly linear with distance at great distances inside the woods, but this attenuation per distance is small. However, close to the edge there is a sizeable boundary effect where much of the incident energy is backscattered away from the forest. In the pine an 8000 Hz tone was reduced by 80% of the value measured at 200 ft. in the first 100 ft. A similar result is expected for the foliage of the corn. Although our data for Ae versus plant density certainly cannot be equated with Ae versus distance at constant plant density, the trends will obviously be the same, i.e. we expect sizeable attenuation in the first 30 to 60 feet and reduced attenuation thereafter. Further work is needed to evaluate this boundary effect. Clearly, this is important for the ultimate prescription of the most efficient vegetative sound screen.

Foilage, stems and soil all reduce sound transmitted near the ground, and together their effects span most acoustically important frequencies. Trucks starting up from toll booths emanate considerable energy at low frequencies. The best way to reduce this annoyance is a narrow band of soft ground very near the source, because once the low frequencies have traveled beyond the point where the ground is effective, they travel great distances suffering only the meager reduction of 6 dB per doubling of distance. On the other hand, high speed traffic generates higher frequencies that are better reduced by dense foilage. For example, a 1000 Hz tone will be reduced in loudness by some 50% more when it passes through 100 ft. of corn or some other dense foliage rather than over open ground. To achieve this same reduction in loudness by distance alone, an observer must be about four times farther from the source. We have used highway noise as an example, but, clearly, these results hold for the same sound frequencies traveling between nearby houses. It seems then, that in congested areas where space is a problem but a natural environment sought-after, plants do offer more than just the psychological advantage gained by screening the view of the source of noise.

Valley Laboratory

(Continued from page 3)

agriculture in the Connecticut River Valley. These have included work on blue mold disease, cyst nematode, air pollution damage, tobacco curing, root patterns in relation to tillage, plant water use, new fertilizers, insect control and behavior, mechanized harvest schemes, weed control in nursery stock and turf, mixes for container nursery stock, potato insects and diseases, tomatoes, and even cucumbers.

In 1965 we changed our name to Valley Laboratory and continue to serve as a research facility for all of the Station staff. We also serve growers, residents, and businesses of North Central Connecticut as a source of information and an open door to the whole Station.

New Publications

Results of Station research commonly appear in journal articles and in Station publications. Bulletins and Circulars listed below are now available on request to Publications, Box 1106, New Haven, Connecticut 06504.

Entomology

B 717 *Chemical control of German cockroaches in urban apartments.* (reprint in press). R. Moore.

C 241 *Control of borers in trees and woody ornamentals.* J. C. Schread.

C 242 *Control of the Eastern spruce gall aphid.* J. C. Schread.

Ecology

C 238 *Candlewood Lake: A tentative plant nutrient budget.* C. R. Frink.

Genetics

C 239 *Virus protection of late-summer squash with aluminum mulch,* W. L. George, Jr., and J. B. Kring.

C 240 *Laurel selections from seed.* R. A. Jaynes.

Report on Inspections

B 719 *75th report on food products, 1970.* J. G. Hanna.

B 720 *Pesticides report, 1970.* J. G. Hanna.

B 721 *Feeding stuffs report, 1970.* J. G. Hanna.

B 722 *Commercial fertilizer report, 1970.* J. G. Hanna.

The Few Feed Many

James G. Horsfall

Director

As a city man, but an erstwhile farmer, I look with a little unease at the elementary statistic that nation-wide 40 of us city dwellers depend upon one farmer for our food. This shows how important agriculture is to an industrial society and that as the number of farms declines, the importance of agriculture rises.

If we balance food produced in this State against food consumed here, we find that 700 Connecticut citizens depend on one Connecticut farmer for milk, meat, and eggs; 3,500 people depend on one farmer for fresh vegetables, and 14,300 depend on one farmer for potatoes.

It is in this context that one reads the following five paragraphs that describe agriculture in our State. They were written last year by a distinguished non-farming citizen of Connecticut for the Report of the Governor's Committee on Environmental Policy.

"Without food we starve, and without agriculture we would have to scrounge among the roots and rocks for sustenance.

"But 'Man doth not live by bread alone' and agriculture provides more than bread. Agriculture occupies two out of the three million acres in Connecticut. It covers our rolling countryside with forests, farms, and open spaces. Its red barns and silos, meadows and maize, lend interest for landscape painters to paint and for all to enjoy.

"Agriculture includes the wooded land, the suburban lawns, the flowers and trees, the rocky ridgetops, the swamps, marshes and shellfish grounds as well as our working farms.

"And so agriculture gives a sense of beauty and pleasure. It may be the pleasure of a beautiful view. It may be the satisfaction, a very special pleasure, of work accomplished on a farm or in a garden. It may be the satisfying taste of Connecticut fresh milk, of homegrown fruit, or sweet corn fresh from the summer field, of tomato's lush ripeness fresh from the vine. This we have known and cherished. Unless we decide wisely now, these pleasures and these satisfactions, these treasured values of our land, could and no doubt will vanish.

"Further, agriculture is the steward of the soil, which man has used since the beginning of time to cleanse wastes from his environment. On this soil grow plants that absorb vast quantities of air pollutants and release oxygen. The plants take up water pollutants as well."

As a result of its concern for agriculture in Connecticut, the Governor's Committee made several recommendations. No. 23 asks that state development be "channeled in a way as to conserve valuable resources needed for future use, i.e., agriculture, fisheries," etc. No. 29 says, "Because agricultural open space is one of the most attractive features of the landscape and some agricultural land must be preserved for future food supply, the state should keep agriculture alive. Tax abatement . . . is not in itself capable of accomplishing this, and we recommend a study of additional ways to keep the farmer in business. The total farm problem—social, economic, and environmental—should be considered."

Potassium Pump

(*Continued from page 2*)

stomata was sufficient to provide the solute needed to increase guard cell turgidity.

These findings therefore provide direct evidence that stomatal opening is controlled by active transport and accumulation of potassium in the guard cells. Movement of potassium (and an accompanying anion) into guard cells is thought to be caused by a potassium ion pump. The energy required to operate the pump may be supplied by metabolic processes that take place in the guard cells in light.

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Bruce B. Miner, Editor

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