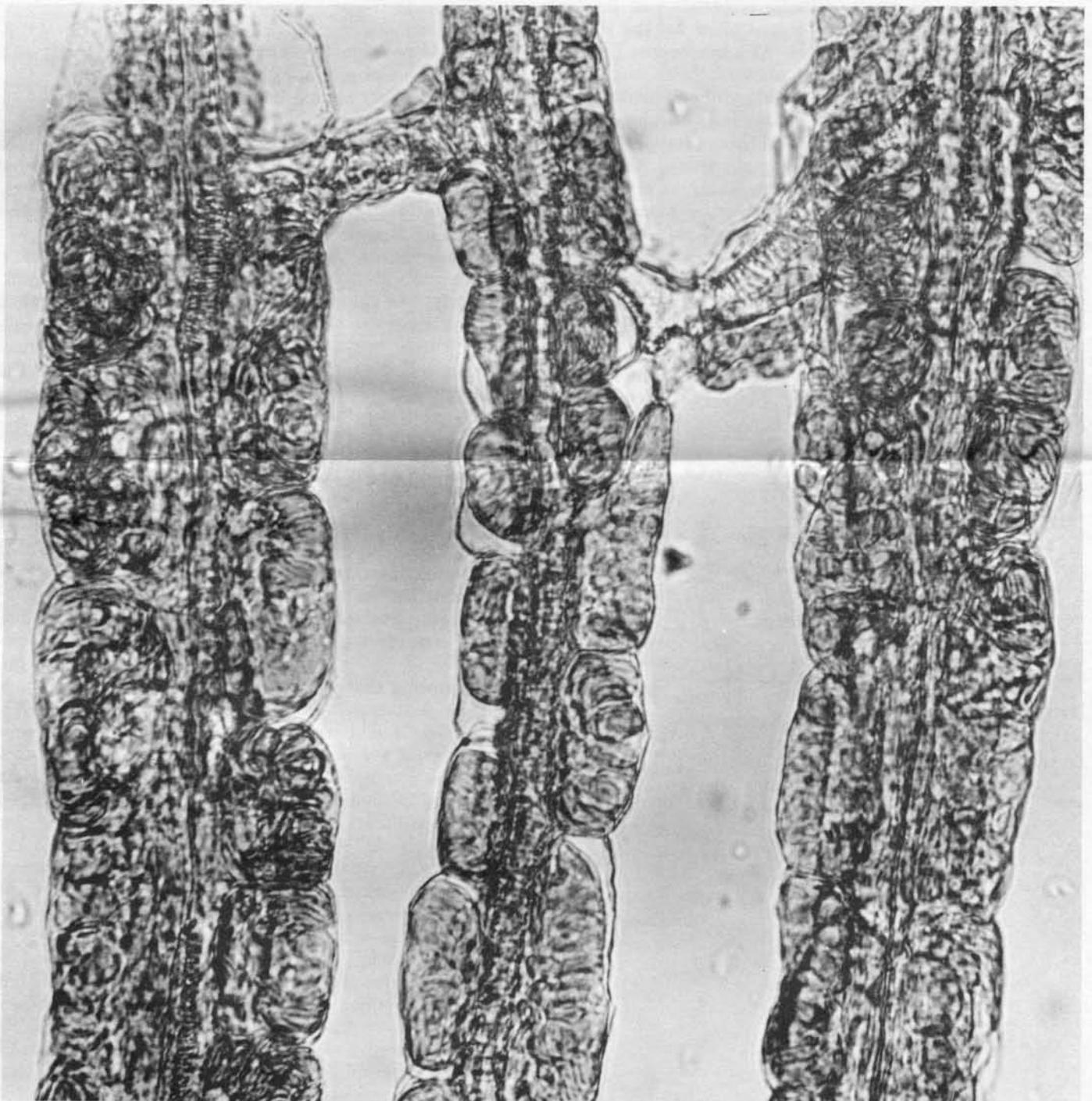


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THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION NEW HAVEN

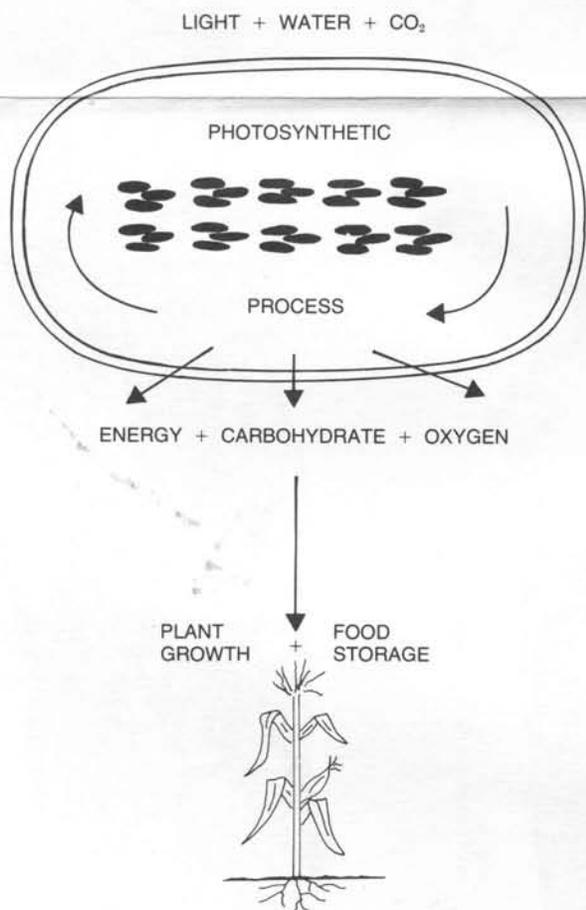
Chloroplast membranes and photosynthesis—

By Raymond Poincelot

PLANT growth is a fascinating phenomenon controlled by a well-integrated series of biochemical processes. One of these processes, photosynthesis, produces food and energy from light, water, carbon dioxide, and minerals (Fig. 1). These photosynthetic reactions take place in the chloroplast, a small green body in leaves.

The flow of photosynthetic materials and products through the chloroplast is controlled by the double-layered envelope membrane. As a biochemist I believe plants which have efficient photosynthesis might have enhanced ability to pass photosynthetic materials or products through their chloroplast envelope membranes. Accordingly, I selected three crop plants which differ in their photosynthetic efficiency, morphology (form and structure), and certain biochemical processes. These plants are spinach, sunflower, and corn in order of increasing photosynthetic efficiency. My hope is that knowledge about and eventual alteration of the properties and functions of these envelope membranes will

Fig. 1. Schematic diagram of the chloroplast and its relationship to the photosynthetic process.



The envelope membranes exhibited different degrees of permeability for materials used or produced during photosynthesis.

make it possible for spinach or other inefficient crop plants to be as productive as sunflower or corn.

Chloroplasts are easily released by grinding leaves in a chilled Waring blender. Chilling prevents destruction of the chloroplasts by leaf enzymes not normally in contact with chloroplasts. Large leaf particles are filtered with cheesecloth and then the chloroplasts are concentrated as a green pellet by centrifugation at 2000 times the force of gravity.

Corn leaves, unlike spinach and sunflower leaves, have a second type of chloroplast near the larger veins. This type of chloroplast cannot be released by grinding alone, since the fibrous nature of the leaf resists grinding. Partial enzyme digestion of the corn leaf produces fibers called bundle sheath fibers (cover photo). The chloroplasts are easily removed by gently grinding these fibers.

Although isolation of chloroplasts proceeds by established procedures, no satisfactory methods were available to isolate their envelope membranes. To overcome this I used osmotic shock. Isolated chloroplasts are suspended in a solution containing low concentrations of salts. Since the chloroplasts contain salts at much higher concentrations, water crosses the envelope membrane to equalize the osmotic pressure. The increased pressure ruptures the chloroplast and the soluble contents spill out, leaving an empty chloroplast envelope membrane.

Knowing that sucrose gradients are useful for membrane purification I developed a gradient to purify the envelope membranes. This gradient consists of three concentrations of sucrose, which are able to float upon one another because of differences in density. The suspension of chloroplast envelope membranes and their leaked contents is placed on top of the gradient and centrifuged at 78,000 times the force of gravity. The intact chloroplast envelope membranes are separated from the leaked contents of the chloroplasts because their differences in density cause them to stop at different densities of sucrose.

At this stage purified envelope membranes can be removed from the gradient as a milky band. The yield is low, when one considers that the yield of membranes is only about 3/100,000 of the original weight of the leaves. Since an average spinach leaf contains about 200 million chloroplast envelopes, the final yield is about 6,000 envelope membranes per leaf.

Electron micrographs of isolated envelope membranes show that their size and appearance is the same as in the intact chloroplast. I used assays for enzymes and chlorophyll found inside the chloroplast to show that the envelope membranes are pure. The hole formed during the osmotic shock is sealed as the envelope membranes swell or contract when the external concentrations of salt are varied.

My first thoughts about early preparations of envelope membranes were that composition may be related to differences in their permeability or ability to pass biochemicals. Therefore, I looked for variations in their lipid (fatty substances), fatty acid, hexosamine (an amino sugar), and protein composition. I found no important variations. Glycolipids (sugar-containing lipids) and phospholipids (phosphorus-containing lipids) were the major and minor lipids, respectively, in all the envelope membranes.

Looking further, I found the types of fatty acids in the envelope membranes were similar. The amounts of some of the fatty acids varied considerably, however, which meant that the fatty acids in the membranes differed in their degree of polyunsaturation. This difference affects the stability of the membranes at different temperatures. My studies indicate that corn envelope membranes should be less affected by higher temperatures than those of spinach and sunflower.

I examined the patterns of the polypeptides (smaller units which collectively form a protein) that comprise the proteins of the envelope membranes in collaboration with Dr. Evelyn Havir. We were happy to see differences in the polypeptide patterns since they may reflect variations in the structural proteins or types of enzymes. I knew that these factors, which can influence permeability and enzyme activity, might directly affect photosynthetic efficiency. I also found differences in the hexosamine content among the various envelope membranes. Hexosamines are associated with proteins and undoubtedly would vary, since there are differences among the proteins.

Some enzymes have been implicated in aiding membrane passage or transport of materials in other biological systems. I was excited to find that one of these, magnesium ion-dependent nonlatent ATPase, is present in the envelope membranes. Corn and sunflower envelope membranes had rates of this enzyme that were 50% and 100% higher, respectively, than spinach; this suggests the transport potential of the corn and sunflower envelope membranes should be greater.

I could only find this enzyme in the envelope membrane portion of the chloroplast. Therefore, this enzyme could be used as a "marker" for the envelope membrane. As the envelope membrane becomes purer during the course of isolation, the activity of this enzyme per unit weight of membrane protein increases.

The permeability properties of the envelope membranes in bacteria have been studied for years. I modified these bacterial methods to determine the permeability of the envelope membranes to various biochemicals required or produced by photosynthesis. Envelope

membranes suspended in a sugar-salt solution maintain their spherical shape and are in an aqueous environment similar to that in the plant. Biochemicals labeled with radioactive carbon were added to these suspended membranes from plants. The experiments were terminated by rapid filtration through a fine filter. The radioactivity retained per mg of envelope membrane protein at different times indicated the permeability of the membrane.

The envelope membranes exhibited different degrees of permeability for materials used or produced during photosynthesis. For example, one study showed sunflower and corn envelope membranes were more permeable than spinach membranes to high concentrations of carbon dioxide, the main photosynthetic substrate. I plan to test other photosynthetic substrates and products.

Eventually I may show that some or all of these differences relate to greater photosynthetic efficiency and greater plant productivity, and that this knowledge may be used to modify plants for greater crop yields.

Fig. 2. A step in the process of removing chloroplasts from corn leaves.



Land use, plant nutrients and water quality

By C.R. Frink and W.A. Norvell

ABOUT 10,000 years ago when the last glacier retreated, Connecticut was left a legacy of lakes and ponds scattered over its landscape. Although no one knows how many there were, we can be sure that there are fewer today. This is so, simply because water runs downhill, carrying with it suspended sediment that helps to fill lakes, and dissolved plant nutrients such as nitrogen and phosphorus that encourage the growth of weeds and algae. Thus, lakes slowly become shallower and more fertile, eventually becoming swamps and bogs. Most of the inland wetlands that we cherish today are monuments to this aging process.

Since many of our lakes are still here—in fact there are about 1000 in Connecticut large enough to have names—it is evident that in nature this aging is generally slow and is usually measured on a geologic time scale. Thus, our concern as we view our remaining lakes is simply this: is man accelerating the aging of these lakes and, if so, what can be done about it?

In seeking answers to this question we have examined lakes in Connecticut from Alexander to Zoar, measuring their chemical and biological properties and attempting to relate what we found to the various kinds of land use in their watersheds. Detailed results of our lake studies are reported in Station Bulletin 759, and we summarize the main findings here, along with some observations on growth-limiting nutrients and the effects of land use. Many aspects of this work await further analysis.

We began during 1973-1974 with an extensive study of the water chemistry, weeds and algae of 23 Connecticut lakes. The lakes, distributed throughout the state, provided a wide range of water and watershed characteristics. An additional benefit was that most of the lakes had been surveyed in 1937-39 by the Connecticut Board of Fisheries and Game, thus permitting comparisons between conditions measured a third of a century apart.

Our first objective was to determine the quantitative relationships between dissolved plant nutrients and the growth of weeds and algae in our lakes. We found a relationship between algae (as measured by the amount of the green pigment chlorophyll) and phosphorus in the water of all 23 lakes as shown in Figure 1. Clearly, as phosphorus increases, so do algae. Of equal importance, the observed relationship implies that removal of phosphorus from our waters should decrease algal growth. The line drawn through the data was fitted by statistical analysis and accounts for about 80% of the observed variability in algae.

Algae also require nitrogen and other plant nutrients for growth as do most higher plants. We found that we could improve our estimates of algal concentrations

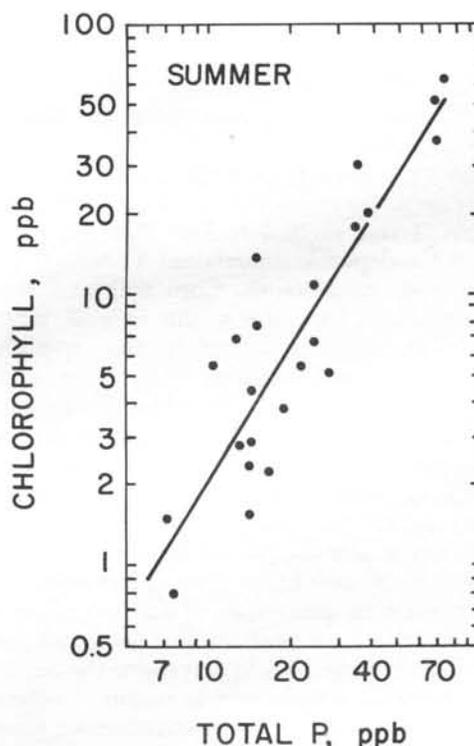
Is man accelerating the aging of these lakes?

slightly by including nitrogen in our prediction equations, but other nutrients appeared to be in adequate supply.

As algae in the water increased we found that transparency of the water decreased markedly. Algae were the main cause of reduced transparency, but natural color and turbidity in these lakes would limit their transparency to about 40 feet in any case. At the height of algal blooms in mid-summer, many of our lakes had transparencies of a few feet or less.

We also collected samples of filamentous algae (*Spirogyra*, *Hydrodictyon*, *Oedogonium*, *Rhizoclonium*) and three common pond weeds (*Myriophyllum* (parrot feather), *Ceratophyllum* (coontail), *Elodea* (waterweed)) from 22 of the lakes. Part of each sample was extracted with boiling water to release easily soluble phosphorus and the remainder was analyzed for total phosphorus (P), nitrogen (N), and potassium (K). The concentrations of these nutrients in the weed tissues were compared to

Fig. 1. Relationship between algae (as measured by chlorophyll concentration) and phosphorus in the waters of 23 Connecticut lakes, 1973-1974.



N, P, and K concentrations that other workers had found limited weed growth. We found that in lake waters that were relatively poor in phosphorus (N/P ratio of more than 30), the growth of most filamentous algae was limited by phosphorus in the water. Only in a few lakes that were relatively rich in phosphorus (N/P ratio less than 20) were most of the algae adequately supplied with phosphorus and had their growth limited by other factors. These analyses of algae support our earlier conclusions from analyses of water that phosphorus is the nutrient most likely to limit the growth of algae in Connecticut's lakes.

In contrast, the rooted pondweeds were rarely limited by phosphorus and only occasionally limited by nitrogen, suggesting that even in nutrient-poor waters, the pondweeds were able to obtain nutrients from the sediments. Out of more than 70 samples of algae and rooted pondweeds, only one had low levels of potassium in its tissues. This indicates that potassium is unlikely to limit the growth of weeds in Connecticut's lakes.

Although many interacting factors influence the fertility of a lake, one of the most important characteristics is the average annual phosphorus concentration in the water. Figure 2 lists the 23 lakes we examined, ranking them in order of their present phosphorus concentrations and comparing these with concentrations measured in 1937-39 (for the 20 lakes for which comparable data are available).

Clearly, phosphorus concentrations have increased in many lakes. As might be expected, we also found that chlorophyll concentrations have increased while transparency decreased in these same lakes. If the recent past is any guide then the future of many of our lakes is in jeopardy.

During the years since these lakes were surveyed in 1937-39, the population of Connecticut has nearly doubled. Housing developments have increased enormously, generally at the expense of agricultural land. To establish quantitative relationships between land use and water quality, D. E. Hill has undertaken a detailed inventory of changes in land use in the watersheds of each of these lakes. The inventory of present land use is based on interpretations of aerial photos taken in 1970 by the Office of State Planning. This inventory is completed and we have recorded 57 categories of land use. These include such variables as residential density, commercial uses, idle land, wetlands, and many others. In addition, we have separated each category into two groups: land within 400 feet of waterways, and land away from waterways. The choice of 400 feet as the dividing line is arbitrary and can be changed if need be.

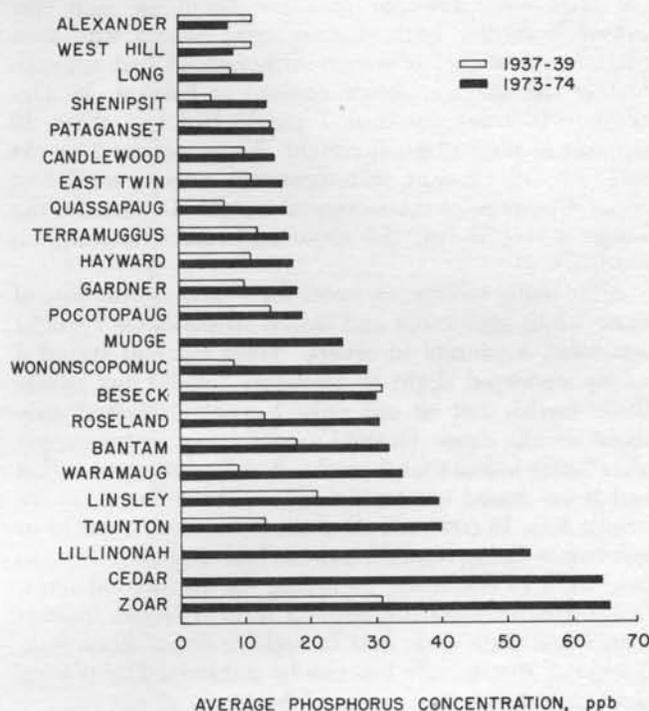
We must also consider the retention time of water in the lake. This is required since large, deep lakes with small watersheds and hence long retention times act as phosphorus traps, removing phosphorus from the water and lowering the expected concentrations. At the other extreme, small lakes with large watersheds and short retention times have little effect on the water flowing through.

Finally, we must consider phosphorus that may be released from lake sediments. Indeed, our studies of Connecticut lakes began with analyses of the sediments, which showed that substantial quantities of plant nutrients, particularly phosphorus, have accumulated in many lakes. Moreover, when lake waters are depleted of oxygen during summer stagnation, we found that phosphorus may be released to the overlying water in large amounts.

In our preliminary analysis we grouped all 57 land uses into four broad categories: residential, agricultural, forested, and other. Each land use was weighted by an appropriate factor for the retention time of the water in each lake. This factor reduces the predicted phosphorus concentrations for lakes with longer water retention times. Since depth of the lake is highly correlated with retention time, depth was not included explicitly in this analysis. Statistical analyses of the data revealed that forested land contributed modest amounts of phosphorus, agricultural land contributed 2 to 3 times that of forested land, while residential land contributed approximately 6 times as much phosphorus as forested land. Because of the diverse uses lumped together as "other" land, this category appeared to contribute little phosphorus.

We are continuing to examine the relationships between fertility of lakes and use of land. We hope to learn to control the process of nutrient enrichment so that future generations can use the land and still enjoy the lakes and ponds of Connecticut.

Fig. 2. Comparison of phosphorus concentrations in Connecticut lakes in 1937-1939 and in 1973-1974.



Keeping Christmas trees fresh

By George R. Stephens and John F. Ahrens

ANOTHER December is nearly at hand and in thousands of Connecticut homes a festively decorated Christmas tree will soon occupy a place of honor. Aside from appearance, the main concerns of many homeowners are needle loss and the flammability of the tree. Our research was directed at providing answers to these concerns.

Why do some cut Christmas trees shed their needles when brought indoors?

Experience suggests that genetic differences and moisture content of twigs and foliage are involved. Pines, true firs (balsam and Fraser, for example) and Douglas-fir generally retain their needles without serious loss after harvest. On the other hand, white and Norway spruces sometimes shed prolifically after being indoors only a few days. However, even those species with normally good needle retention will shed if allowed to dry excessively.

Because white spruce is one of those species that sometimes sheds excessively it seemed a good candidate for experiments. Beginning November 1 and at 2-week intervals until December 13, white spruce trees were cut, bundled, and stored outdoors in a shady pine grove. On December 19 these trees plus some freshly-cut ones were brought indoors. About an inch was sawed from the butt of each tree, which was then placed in a bucket of water. Although all had approximately the same moisture content at harvest, by December 19 trees cut 5 or 7 weeks had lost about 20 percent of their original weight. Trees cut for 3 weeks lost only 10 percent and those cut only a week lost about 4 percent of their original weight. Obviously, the longer a tree is cut, the more moisture it loses, even outdoors.

After being indoors in water for 7 days needle loss of some white spruce cut and stored outdoors for 7 weeks was rated moderate to severe. Trees cut and stored 3 weeks sustained slight to moderate needle loss, while those freshly cut or cut only 1 week exhibited only slight needle drop. Despite a continuous water supply after being indoors for 3 weeks, 1 of 5 white spruce that had been stored outdoors for 7 weeks suffered severe needle loss. In contrast, all of those trees cut 5 weeks or less before being brought indoors had negligible loss. In fact, most of the trees, including the freshly cut ones, had a greater moisture content after 3 weeks indoors than when they were first brought indoors. Therefore, it appears that needle loss can be minimized by placing trees in water as soon as possible.

Cut trees, properly stored outdoors, and then maintained in a continuous water supply indoors, present no real hazard.

What happens if the water supply is interrupted after a cut tree is brought indoors?

White spruce trees cut on December 13 were brought indoors the same day, recut, and immediately placed in water. After 3 days some were removed from the water for 24 hours and then replaced, representing the situation where a tree stand is allowed to go dry but is subsequently refilled. After 14 days indoors in a room at 65°F, needle loss for trees with interrupted water supply was six times greater than needle loss from trees supplied continuously with water for the 14 days. Needle loss on trees with an interrupted water supply also was far greater than needle loss from trees stored dry indoors for 10 days before being placed in water. The effect of temperature on water loss is also dramatically shown in this experiment. Trees stored dry indoors at 65°F for 10 days dropped to the same moisture content as did trees in shaded outdoor storage in 7 weeks!

We may conclude thus far that needle retention on white spruce, a species with a reputation for shedding, can be minimized by selecting a tree cut for the shortest possible time, by recutting the butt so as to remove wood plugged with pitch and dirt, and by placing the recut tree immediately in a continuous water supply. Interrupting the water supply may be worse than no water supply at all.

How much water will a cut tree use indoors?

In general, the larger the tree and the warmer and drier the indoor atmosphere, the more water the cut tree will transpire. The table shows average daily water use by trees of varying size and species maintained indoors at 65°F for approximately 3 weeks. Average daily

Water loss by cut Christmas trees indoors at approximately 60°F for 3 weeks*

Species	Avg. Daily Use pints
White spruce	1-2.3
Colorado blue spruce	1.3
White pine	2.2

* Based on trees approximately 6 ft tall and weighing 20 lbs.

water use ranged from 1 to nearly 2.5 pints. However, initial water consumption was sometimes 2 to 3 times greater than the 3-week average, especially for the larger trees. Obviously, a large reservoir of water helps ensure an uninterrupted water supply to the cut tree.

Is there anything I can add to water to make my Christmas tree last longer?

A continuous water supply will maintain or increase the moisture content of twigs and needles and will help prevent needle loss. However, each year home remedies and manufactured products are touted to aid needle retention or to prolong freshness of cut trees. We have tested four commercial preparations and the pure chemical equivalent of two. Included were a chemically impregnated card, two dry chemical mixtures and a liquid preparation, all designed to be added to the water reservoir of a Christmas tree stand. Tests were conducted on cut branches or whole trees. In brief, none of these additives provided any clear-cut benefit over use of water alone for needle retention or for maintaining moisture content of twigs and needles. Viewed another way, use of additives dilutes the water available to cut trees. For a more complete account of these experiments read Station Bulletin 760, "The Effects of Additives on Freshness and Flammability of Christmas Trees".

Are cut Christmas trees safe to use in the home?

We believe that cut trees, properly stored outdoors, and then maintained in a continuous water supply indoors, present no real hazard. As proof, we held branch tips at the apex of a 2.5-inch flame from a Bunsen burner and observed the time required to consume the needles on one side of the twig or to cause the twig to flare and ignite. Twigs with a high moisture content sputtered and charred until the needles were consumed, but did not burn when removed from the flame. Dry twigs continued to burn when removed from the flame. In all tests the only twigs that supported combustion were some from white spruce trees held dry indoors for 7 or 14 days, some from trees cut and stored outdoors for 7 weeks, and some from trees removed from water for 24 hours and then replaced. Twigs from trees maintained continuously in water indoors charred, but did not support combustion when removed from the flame. Additives had no effect on ignition time of twig samples. The use of flame such as we describe is a severe test, and the marked contrast in ignition times between cut trees maintained in water and such common items as paper and cotton cloth is clear from the table.

Based on our observations and experiments the following appear likely to ensure safe use of cut Christmas trees indoors: Although the tests indicate that cut trees can be stored outdoors for several weeks without deleterious effect, the freshest trees should have the least needle loss because the storage conditions of cut trees cannot always be discerned. Larger trees sometimes require thorough jarring to remove the accumulation of

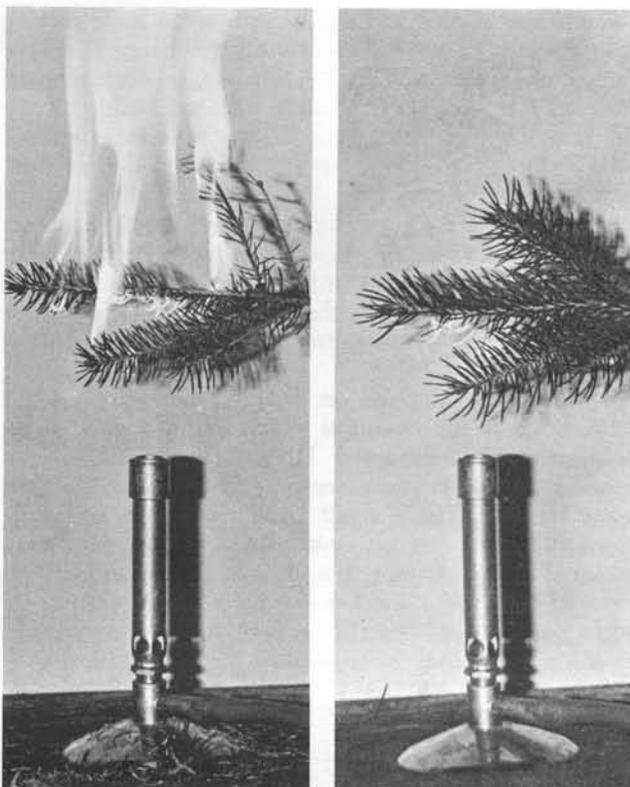
dead needles from interior branches. Recutting the butt and immediately placing the tree in water when brought indoors permits rehydration of twigs and needles. Because larger trees, especially, may initially use several pints daily, the water reservoir should hold at least a gallon to ensure a continuous water supply.

Ignition time of branch tips exposed to a 2.5-inch Bunsen burner flame

Species	Outdoor Storage days	Indoor Use days	Ignition time seconds
White spruce	49	21	11.9*
	7	21	13.0
	0	14	9.9
	0	14	7.1*
(dry for 24 hrs)			
Colorado blue spruce	7	21	18.9
	7	21	12.8
(dry for 21 days)			
White pine	3	22	17.8
Paper, cardboard, cotton cloth			1.5 or less*

* One or more samples supported combustion.

Fig. 1, left, A branch tip that has lost moisture equivalent to 50 percent of its original weight flaming at the apex of a 2½ inch Bunsen flame, representing a tree that has been allowed to stand without water. This branch continued to burn when it was removed from the flame. At the right, a similar branch tip at the apex of the flame chars and sputters but will not support combustion when the flame is removed, representing a tree that has been held in water.



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Plant breeding enters a new age

By Peter R. Day

STATION geneticists are about to explore some entirely new methods for moving genetic information among organisms that we have never been able to cross. The new methods were developed during the last two years by bacterial geneticists. If they can be applied to higher plants, their potential for increasing the yield of food crops will be tremendous. It should be possible in the future to transfer the high rate of photosynthesis of corn to wheat and other cereals, to produce cereals which fix their own nitrogen, to improve the nutritional value of many of our staples and also to produce a wide variety of new forms that we presently do not have. The new techniques will add a new dimension to work already underway at the Station using cultured cells of crop plants to reduce photorespiration, increase rates of photosynthesis and lower the loss of carbon dioxide during the hours of darkness. We are also working to increase the quantities of a limiting amino acid in soybean tissues. Our approach is to modify or delete undesirable features that are present in these crops. The new work in bacterial genetics adds the prospect of being able to introduce entirely new genetic information into these systems.

The methods depend on isolating DNA from plant cells. DNA is the chemical which encodes genetic information. The extracted DNA may be chemically joined to small circular pieces of DNA that are found in many bacteria. These small DNA circles are known as plasmids. They can be isolated and moved from one bacterial cell to another. If a plasmid carries an inserted piece of DNA from another organism and is introduced into a bacterial cell then the genetic information carried by the plasmid may be expressed in the new host cell.

I believe that transfer of genetic information from one plant species to another will help produce the needed abundant and economical food.

Scientists have been able to introduce genes from yeast into a bacterium and to show that these genes are expressed in the new environment. We plan to introduce DNA from soybeans and other plants into a bacterium and examine the bacterial cells for expression of plant genes. Once such cells have been identified and selected it is possible to multiply greatly the number of copies of the introduced plasmid that the cells carry. In this way we should be able to amplify and thus purify certain genes which can then be extracted from the bacteria to be used in testing ways to introduce them into plants which lack them. In short, we seek ways of moving genes from one plant to another by using bacterial cells as a bridge.

Although farmers must grow more food to keep up with increasing population, agricultural scientists must show the way. I believe that transfer of genetic information from one plant species to another will help produce the needed abundant and economical food.

Guidelines for research with recombinant DNA have been developed by a committee of the National Institutes of Health. This committee has concluded that the risks of working with plant DNA are low. We are following these guidelines and in fact are being more conservative in our research.

Frontiers of Plant Science published in May and November, is a report on research of
The Connecticut Agricultural Experiment Station. Available to Connecticut citizens upon request.
Vol. 29 No. 1 Fall, 1976 ISSN 0016-2167 Paul Gough, Editor