

The cover of the journal 'Minnesota Science' features a photograph of purple flowers, likely alfalfa, in a field. The text 'minnesota science' is printed vertically in white on the right side. Below it is a large, stylized white logo consisting of the letters 'M' and 'S' intertwined. Underneath the logo, the text 'volume 32 number 3 fall 1976' is printed. In the bottom right corner, the text 'Breakthrough . . . page 4' is displayed. In the bottom left corner, the text 'AGRICULTURAL EXPERIMENT STATION UNIVERSITY OF MINNESOTA' is printed.

minnesota science



volume 32 number 3 fall 1976

Breakthrough
. . . page 4

AGRICULTURAL EXPERIMENT STATION
UNIVERSITY OF MINNESOTA



Keith Huston, Director of the University of Minnesota Agricultural Experiment Station, and Donald K. Barnes (left) examine a super nitrogen-fixer. (For story see page 4.)

COVER: An experimental strain of alfalfa at the Rosemount Agricultural Experiment Station.

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New Support for Research - Aid From Minnesota Producers, Processors, and Suppliers

KEITH HUSTON
Director,
University of Minnesota
Agricultural Experiment Station

PRINCIPAL financial support for experiment station research traditionally has been public funds. It is likely that such support will continue because the benefits of research are enjoyed by all Minnesotans through the users of research—farmers; owners of agricultural and forest lands; and other producers, processors, and suppliers.

But, other and new sources of support are important, too. This year, 18 different organizations of producers, processors, suppliers, and associations of professionals will fund experiment station research. Others are planning support for next year.

Four producer groups—The Minnesota Soybean Research and Promotion Council, the Minnesota Turkey Growers Association, the Minnesota Beef Research and Promotion Board, and the Red River Valley Potato Growers Association—have been major supporters of experiment station research. Also, one processor group, the Malting Barley Improvement Association, has been a major supporter. Members of each group assess themselves to secure monies for research. Although none of the groups provides enough resources to completely support one full time scientist, each provides sufficient funds to strengthen research of several scientists. Those funds are used to buy important pieces of expensive equipment, or to support a young Minnesotan

entering a research career, or to furnish additional help with experiments. Support from other groups, though smaller, is important and used similarly.

Most groups choose the research they want to support: Some choose the scientist who is to receive their support. And some limit the ways in which funds are to be spent. In most cases, the groups share planning with administrators of the University of Minnesota Agricultural Experiment Station. The Minnesota Turkey Growers, through their Research Committee, chose as their highest priority research areas—improving nutrition, semen preservation, and fertility; and controlling viral enteritis, respiratory diseases, and mycotoxicoses. The Red River Valley Potato Growers concentrated their support on developing and testing adaptability, quality, and disease resistance of new potato cultivars so as to speed variety development. The Malting Barley Improvement Association wanted to hurry the development of new, better quality, and disease resistant varieties. The Minnesota Golf Course Superintendents' Association needs to control turf grass diseases. The Minnesota Nurserymen's Association wants new and better ornamental shrubs.

Some groups make special grants for purchase of special equipment. The Minnesota Soybean Research and Promotion Council helped in the purchase

of a calculator, planter, growth chamber, plot combine, and a special protein analyzer. These help our experiment station soybean breeder to develop better quality high-yielding soybean varieties faster.

Farmers and others who use research results from the University of Minnesota Agricultural Experiment Station think that their support encourages a broader exchange of ideas between users and scientists. This exchange of ideas helps scientists to focus more sharply on important research areas. On the other hand, a Washington-based consumer interest group recently expressed concern about publicly funded scientists accepting such support, for fear that such support might compromise the scientist's objectivity. Fortunately, funds are accepted by the experiment station only when a simple contract, or memorandum of understanding, has been agreed upon by the donors and the station. Station policy requires that these agreements must protect the scientists from interference and protect their rights to publish all findings.

Scientists and Administrators of the University of Minnesota Agricultural Experiment Station think that support, big and little, from these Minnesota producers, processors, and suppliers has contributed significantly to the station's ability to respond to high priority state needs. □

Breakthrough in Alfalfa Research

PHILLIP E. MILLER

Notice the nodules and fibrous structure of the roots of this alfalfa plant. These are traits which the UM researchers look for when selecting for high nitrogen-fixation.





Students of the University of Minnesota dig up strains of alfalfa for disease resistant evaluation.

ALFALFA—the Queen of Forages—is being bred into a high-production factory for nitrogen fixation.

Super alfalfa will be the result.

Prospects from this new generation of alfalfa could bring millions of dollars—in terms of improved pastures, improved soil tilth, reduced costs of livestock feed, and fertilizer saved.

Nitrogen is the major limiting nutrient for more than 200 million acres of grassland production in humid areas of the US.

To increase their production, farmers must apply either manufactured fertilizers or else obtain natural fertilizer from forage legumes for their sources of nitrogen.

A few non-leguminous plants fix nitrogen, as do some free-living microorganisms including certain algae, bacteria, and fungi. However, forage legumes such as alfalfa—in combination with the bacteria *Rhizobium meliloti*—are the most effective natural source of nitrogen. They produce tons of biologically fixed nitrogen every year. Since as early as Roman times generations of farmers have used legumes to enrich the soil.

"It is surprising that virtually no US research has been conducted on trying to improve on nature by breeding forage legumes for increased nitrogen fixation," says Donald K. Barnes, USDA research geneticist at the Minnesota Agricultural Experiment Station.

"Research has been conducted on

trying to find more effective rhizobia strains, but not on improving the plant."

For years, alfalfa has been coasting along on its good reputation for nitrogen fixation. Yet, several years ago—after nitrogen fertilizer costs skyrocketed—several national studies on research needs placed nitrogen fixation among the top priority needs for research.

"Most of the recent nitrogen-fixation research has been on soybeans," points out Barnes. "But relatively little research has been done on breeding soybeans for improved fixation. And of the nitrogen-fixation research on soybeans, little has been applicable to forage legume research.

"Our interest in nitrogen-fixation research on alfalfa began prior to the energy crises.

"As we watched the developing energy crisis it looked like there would be difficulties—especially in the third world—in supplying nitrogen fertilizer, especially nitrogen fertilizer, manufactured from natural gas.

"Since alfalfa already had a great capacity to fix nitrogen—250 to 530 pounds per acre—depending on environmental factors and nutrient balances, we thought it should be possible to improve on this."

But new funds for this research were not immediately available.

"Yet, the potential benefits seemed great enough to redefine some of our other priorities," he says.

What would be the benefits of a super nitrogen-fixing alfalfa?

Minnesota geneticist Barnes estimates the potential annual benefits in the U.S. from increasing N_2 fixed per acre by 50 pounds as being equal to \$1,706,000,000—a 5 percent increase in forage production plus 938 million pounds of N_2 at \$0.15 per pound for grass and grain crops.

“An increase in the use of symbiotically fixed nitrogen also will save fossil fuel by reducing the need for manufactured nitrogen,” points out the Minnesota researcher. “The value of alfalfas with such increased nitrogen-fixation potential would be even greater for many third world countries where cash for nitrogen to fertilize grasslands is not now available.”

With rising fuel and fertilizer costs farmers would welcome a super alfalfa—one that would not only grab more free nitrogen from the air but would also fix more of it in the soil, for future crops. Result: good forage, better soil, and money saved with nature’s application

of nature’s nitrogen in contrast with tractor-applied synthetic ammonia, for example.

Current varieties of alfalfa lose most of the nitrogen they fix when upper portions of the plant are removed for livestock feed.

We wanted to increase the residual amount available in the soil following alfalfa, says the University of Minnesota geneticist.

“Progress in our program was enhanced when Mark Seetin—presently a farmer from Winnebago, Minnesota—undertook research (for his Master of Science degree) on nitrogen fixation in alfalfa,” says Barnes. “He set out to develop a procedure for selecting plants with exceptional N_2 fixation potential when exposed to a mixture of rhizobia. This is an important consideration because evidence, in soybean, indicated that it was nearly impossible to select outstanding rhizobial strains that could

effectively out-compete naturally occurring soil rhizobia.”

Seetin and Barnes set out to select for a high nitrogen-fixers which would be compatible with many different strains of rhizobia—bacteria that live in root nodules and fix nitrogen.

The soil bacteria enter the plant through root hairs. The root hair cells react to such an invasion by forming a wall—an infection thread—around each bacterium.

Secretions from the bacteria stimulate an abnormal growth of root cells. The infection threads enter these stimulated cells.

Finally, the enclosed threads burst and release the bacteria which stimulate more growth and development of the root cells. The dividing and enlarging root cells form a swelling, or nodule, on the root.

How is nitrogen gas from the air captured?

Minnesota researchers Duane M. Smith, Donald K. Barnes, Laddie J. Elling, and Fred I. Fosheiser (left to right) evaluate alfalfa strains for bacterial wilt resistance.



"Fixation of molecular nitrogen is a complex system requiring energy-yielding metabolic activity of the plant, electron transport systems, and ammonia-assimilating systems," says the Minnesota plant geneticist.

The bacteria use nitrogen to make ammonia which the plant uses for growth. In return, the bacteria get food and water from the plant. Neither the plant nor the bacteria can alone fix nitrogen. It is the symbiotic relationship which makes alfalfa, and other legumes, nitrogen fixers.

How do you tell a good fixer from a poor one?

"Measurement of the nitrogen fixation potential of leguminous plants was difficult until recently," says Barnes.

In the late 60's, scientists developed an acetylene reduction technique that accurately estimated the amount of nitrogen being fixed by a plant. In this system nodules take in acetylene and reduce it to ethylene. When measured on a gas chromatograph the amount of ethylene is in direct proportion to the amount of nitrogen being fixed.

"Seetin standardized the system so it could be used on alfalfa grown in the greenhouse," explains Barnes.

The UM researchers analyzed more than 2,000 alfalfa plants. From these they selected those plants with the highest, and those plants with the lowest, nitrogen-fixation rates.

After crossing these plants they planted the resultant seed in plastic tubes placed in a bench of sand. They examined the crosses for root type, nodulation, and acetylene reduction—an indication of nitrogen-fixing abilities.

The results to date are exciting," says Barnes.

"When we looked at the characteristics of high nitrogen-fixing plants we found many of them had a fibrous type root system with many nodules. Other internal factors were also important.

"Crosses between parent plants selected as high nitrogen-fixers had more than 2 times greater nitrogen-fixation rates than crosses between plants selected as low fixers."

"Crosses between high and low parents produced intermediate progeny."

The next major sequence in the application of this research will be taking these findings from the greenhouse and applying them to field test plots of the super fixer of nitrogen.

The breakthrough is one of knowledge and methodology: the knowledge being that the lid is off previous beliefs of nitrogen-fixation limits. The methodology is the means to measure rates of nitrogen fixation for alfalfa.

How does the super alfalfa calendar—from conception to delivery—read?

The next step is to determine if the new alfalfa strains will give similar performances in the field.

"This will take at least 2 years," says Barnes. "Anticipating positive results, we have already begun a program to develop a new variety with superior nitrogen-production potential.

"If we are successful in developing this new type of alfalfa it would offer many possibilities for new agricultural practices that are now impossible or impractical.

"Probably the first use of a new variety would be to interseed it into grass pastures to increase the productivity and quality of the forage. The new development of interseeding improved forage varieties into pastures is one of the most important breakthroughs in the history of forage production.

"The ability to further increase the level of symbiotic nitrogen would further enhance the value of this breakthrough.

"Another possible use of a super nitrogen-fixing variety would be the development of intensive annual cropping systems. It should be possible to plant alfalfa with small grains, harvest this for silage after about 70 days, and then produce one or two harvests of alfalfa hay by October 15. I would hope that nitrogen fixed in the soil could then provide 100 pounds of nitrogen per acre for corn the next year. This type of intensive cropping would be best adapted where late summer irrigation could be used."

The great potential for high nitrogen-fixing forage legumes can only be imagined at this stage. But the research being conducted at Minnesota is bringing it closer to reality.

Seetin points out that, "It is imperative that nitrogen-fixation research be increased on forage legumes as a method for utilizing the full potential of forages in the production of meat and milk."

The Agricultural Research Service of the USDA also believes in this philosophy because it has just increased its support of this research at the Minnesota Agricultural Experiment Station. □

MINNESOTA has been prominent in the national history of alfalfa. Alfalfa was introduced to the state in 1860 when an immigrant named Grimm brought a strain from Germany to Carver County, Minn. The original strain was not adapted to the area but by saving seed from the surviving plants, Grimm increased its cold resistance. Grimm alfalfa was distributed—with the help of the Minnesota Agricultural Station—to farmers all over the northern US. Now, thanks to this beginning, the state ranks second in the nation in hay production. In good years some 6,500,000 tons of hay—alfalfa and alfalfa mixtures from 2,200,000 acres of Minnesota cropland bring to the state valuable feed for milk cows, which rank third in the nation, to produce milk, which ranks fourth in the nation—compared to the agricultural production of other states.

Alfalfa is insect pollinated and attracts large bee populations. These bees collect large quantities of nectar from alfalfa, and help bring Minnesota honey production to fourth in the nation.

"UM agronomist Laddie J. Eiling started alfalfa breeding research at the Minnesota Agricultural Experiment Station in 1950," points out Donald K. Barnes, USDA research geneticist at the Minnesota station.

"During the last 25 years the cooperative efforts of scientists in the Department of Agronomy and Plant Genetics and the Department of Plant Pathology of the College of Agriculture have been responsible for increasing the awareness of producers and breeders to the importance of disease resistance."

Barnes; Eiling; and Fred Frosheiser, USDA Research Plant Pathologist, have evaluated essentially all US and Canadian varieties for bacterial wilt resistance and Phytophthora root rot resistance. They also have been responsible for the development of 2 new alfalfa varieties.

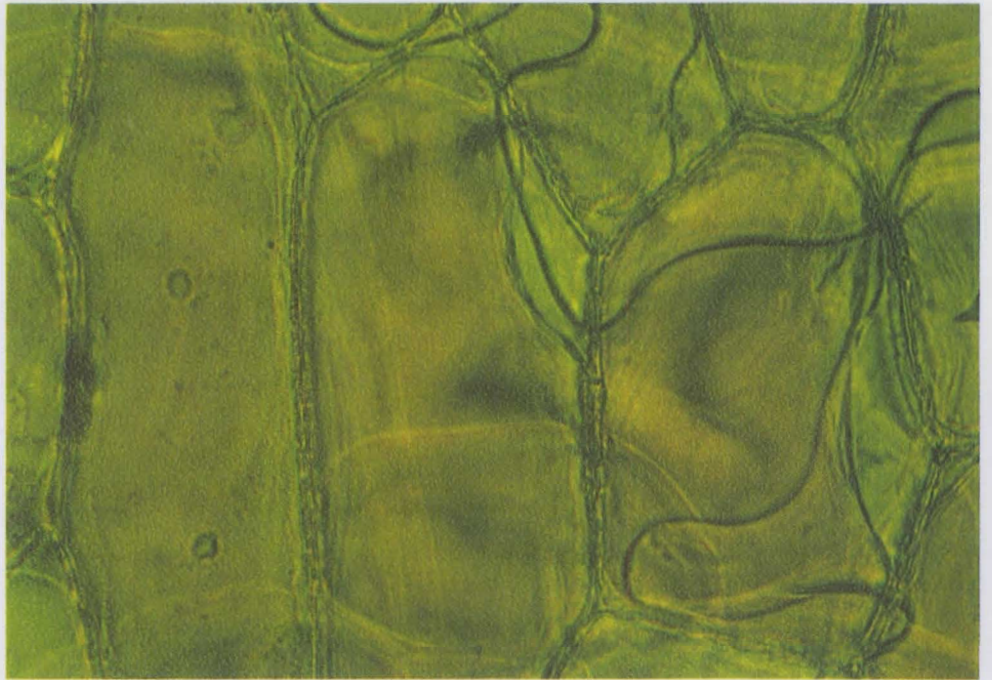
In 1974, seed of Agate was released by the Minnesota Agricultural Experiment Station. It was the first mid-west adapted alfalfa variety with a high level of Phytophthora root rot resistance.

In 1976, seed of Ramsey became available. Ramsey—a very winterhardy, multiple pest-resistant variety—has been released for use in long-term stands of 5 or more years. It should be especially well adapted for interseeding in sod.

"The Minnesota alfalfa breeding program is an excellent example of how cooperation between the Agricultural Experiment Station and USDA scientists results in better varieties for the farmer," says Barnes. □

Onions Survive Fre

Here are onion epidermal cells as seen under the microscope while the onion cells are freezing. The very thin ice front is seen as the curved line.



After installing thermocouples—to indicate temperature—in these onions, UM experiment station researchers froze the onions to -14°C . The thawed onions, seen here, have a soaked appearance of the outer scales. This appearance is caused by "infiltration of the tissue."



These onions were frozen to -21°C and then thawed. Notice the discoloration which indicates water "infiltration" of the tissue.

zing

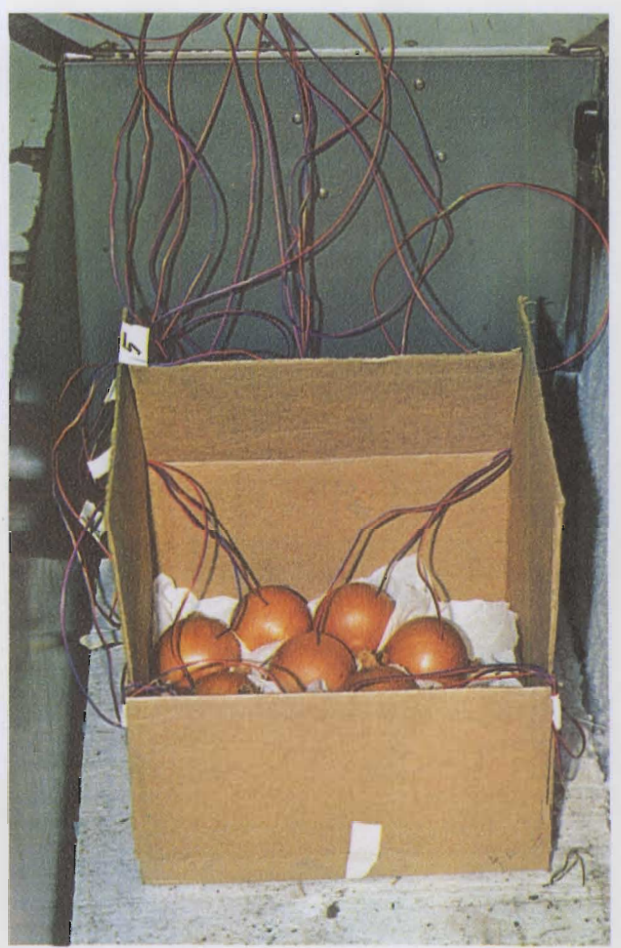
ON'S TENDER tissue survives rather than freezing.

After a Minnesota fall brought 5 consecutive nights of 0°C temperatures at the University of Minnesota Agricultural Experiment Station test plot, the sixth night brought a low of -10°C. UM's arch onions were frozen in.

Thus began the latest Minnesota study by horticulturists Jacob Levitt and Jiwan

P. Palta of cold hardiness of onions. "I was surprised when I thawed the onions and they were not damaged," recalls UM horticulturist Palta.

"We thought those harvested onions would get soft and watery. But we brought them back anyhow. To our surprise most of them came out in good condition. So we worked on methods of reducing freezing stress."



Minnesota researchers put these onions in a cardboard box which they then placed in this freezer to get accurate temperature control during their studies of the freezing process in onions.

Downing Yellow Globe onions in an experiment station plot at Elk River, Minn. Minnesota plant physiologists later harvested these onions for their work on cold hardiness of plants.

What is the best way to thaw frozen onions?

"If the freeze is at -4°C , then we can keep the onions frozen for 2 weeks," says Palta. "But then they have to thaw slowly over ice or at 0°C . It takes about 12 hours to thaw them without injury."

How do onions freeze?

Due to their high water content and large size, it takes a relatively long time to freeze whole onions once freezing has started.

"We found that it takes a temperature of about -4°C for 5 days or -11°C for $1\frac{1}{2}$ days to freeze onions to the respective temperatures," says Palta.

Onions exposed to -4°C cool down to about -3°C in about 19 hours.

"Frost on the onions does not penetrate to the living, internal tissues, because of the waxy, dry, surface," says Palta. "The bulbs can therefore remain supercooled for hours, at sub-freezing temperatures."

After an onion is first exposed to -4°C and starts freezing, it takes about 5 days to freeze the whole onion down to a temperature of -4°C , explains Palta.

Onions exposed to -4°C remain supercooled at -3°C for at least 20 hours before they start to freeze. As freezing starts, the onions warm up to about -1°C (due to the heat released by the freezing water) and remain there for about 7 hours. Then the onions slowly get colder and finally reach about -4°C in about 120 hours after nucleation—the start of ice crystal formation.

When the Minnesota scientists exposed onions to -4°C for 5 days, they found that only about 10 percent of the onions nucleated spontaneously—started freezing—while 90 percent remained supercooled and had to be nucleated to initiate freezing.

When they lowered the temperature 5°C every 4 hours, they found that the onions cooled 1°C per hour.

Onions exposed to -11°C cooled down to about -4°C and in 3 hours started freezing spontaneously. Once freezing starts, the onions warm up to about -1°C and remain there for about 7 hours. Then the onions cool to -11°C within about 35 hours after nucleation.

How do onions thaw?

After frozen onions are put in a box to thaw they warm up very rapidly, to about -2°C in the case of -4°C treatment and to about -3.5°C in the case of -11°C treatment. During the next 10 hours the onions thaw very slowly, warming up from -2°C to 0°C and from -3.5°C to -0.5°C in the 2 treatments, respectively.

In general it took about 12 hours to thaw onions irrespective of the temperature to which they were frozen.

At each temperature of -4 , -9 , -15 , -20 , and -25°C , Palta took out a different pair of onions and, each time, thawed the pair over ice. He halved the thawed onions and then checked them under the microscope. He looked for freeze-damage such as infiltration of the onions tissues. He examined part of the onion scales for signs of life: protoplasmic streaming (movement) and occurrence of plasmolysis (protoplast shrinking) in salt solutions.

Damage to the tissue increased as the freezing temperature decreased.

The type of damage which the Minnesota scientists call "infiltration of the tissue" has been interpreted by some as a consequence of breakdown of the semipermeability property of the cell membrane. Such breakdown would cause cell fluids to leak into the spaces between cells. The percent of tissue infiltrated was thought to be correlated with the percent of dead cells in the tissue.

The Minnesota Agricultural Experiment Station workers find that this interpretation is incorrect.

The UM researchers thaw and cut up onions and leave them at 5°C and 25°C to see if injury increases or decreases with time. In onions frozen to -4°C and -9°C infiltration disappears in 3 days. In onions frozen to -15°C , infiltration disappears from all the scales except the first 2 scales which remain infiltrated.

"There is no relationship between the extent of infiltration and number of dead cells in the thawed tissue," says Palta. "Infiltration of tissue increases from 30 percent to 100 percent as the freezing temperature is lowered from -4°C to -20°C whereas almost all the cells survive.

"This shows that whatever injury occurred to the cells—which led to the infiltration of the tissue—could be reversed, automatically in the case of -4°C , -9°C and to a large extent in the case of -15°C but not in the cases of -20°C and -25°C . Onions kept at 5°C recover much better than those kept at 25°C . Further, most of the cells survive from temperatures as low as -20°C ."

At -25°C almost all the onion cells die.

"Epidermal cells cooled and frozen at the rate of 1°C per hour survived as low as -20°C ," explains Palta. "About 60 to 80 percent of the cells were living at this temperature. At temperatures close to -21°C or -22°C most cells appeared dead and at temperatures warmer than about -18°C all cells were living. Although there was a little

variability between different onions, in general most epidermal cells survived up to -20°C . Later studies with faster cooling rates up to 4°C per hour gave the same results."

Why do the cells die below -20°C ?

"Our studies with onion bulb cells indicate that the damage starts at the cell membranes," says Palta.

"Evidence has been found that the active transport systems (the biochemical pumps) located at the cell membranes are damaged first."

Does damage increase if onions are kept frozen for several days?

"Yes, immediately after thawing the extent of infiltration of onion scales increases with increased freezing periods from 6 to 12 days," says Palta.

"In general, infiltration is much higher at -11°C than at -4°C . However, onion cells remained alive even after having been kept frozen up to 12 days at a temperature of -11°C ."

Is repeated freezing and thawing especially damaging?

"No effect of repeated freezing and thawing was found on the extent of infiltration at a freezing temperature of -4°C ," says Palta.

Onions frozen 1, 2, and 3 times showed about 30 percent tissue infiltration which disappeared in all cases within 3 days after thawing.

Is this information of use to the home gardener, or onion farmer, with onions frozen in the ground?

"Just because onions are frozen in the ground doesn't mean they are dead," points out Palta.

Gardeners should wait for one warm day before digging up previously frozen onions, say the UM scientists.

So wait a day and thaw at about 2°C .

"There are several things to watch out for if cold temperatures are on the way and you still have onions in the ground," says Palta.

"Do not disturb onions cooled below freezing temperatures as disturbance could start nucleation, and freezing.

"Do not dig out onions frozen in the field and then bring them to a warm room. Instead, they can be transferred to a place with 2°C for at least a day.

"If onions freeze down to about -10°C , do not leave them in the field for a long time.

"If they are frozen at -4°C , they can be left at that temperature for up to 12 days with no damage if thawing is then done for a day at 2°C ." □

Agricultural Chemicals and Human Health

RUSSELL S. ADAMS, JR.¹
Department of Soil Science

HERBICIDES AND PESTICIDES help to control weed and insect pests and thereby help to feed the world, reducing malnutrition and starvation.

Yet, some people take stands against the use of manufactured chemicals for food production, considering them a hazard to health.

Hazards and Benefits

Hazards which herbicides and pesticides present to man cannot be considered in a vacuum. Benefits must be weighed against hazards.

Weeds could not be removed from the crop row without herbicides unless considerable hand labor were introduced or unless we returned to smaller, slower-speed machinery. In either case the number of people fed per farmer would be dramatically reduced, production would decline, and our standard of living would drop.

To revert to older practices would render some land marginally productive and result in production loss. Weed control in cereals in the northern sections of the Canadian prairie provinces and parts of northern Minnesota, for example, could only be accomplished by regular cultivated fallow. This would result in a significant loss in production and make cultivation of some land unprofitable.

Reduction of damages from pests increases storage life of food and frequently increases the nutritional quality of the product.

Few realize the demand that pests make on crop production. Losses due to pests of all kinds (weeds, insects, and diseases) accounted for 51 percent of the strawberry crop, 31 percent of the potatoes, 30 percent of the corn, 26 percent of the wheat, and 19 percent of the apple crop in the US during the decade of the 1950's. This is in spite of pest control measures taken.

According to the Mrak report, 41 percent of the rice and 48 percent of the sugarcane that could be grown in Asia are lost to insects alone.²

As a more direct benefit, pesticides have been used to control specific insect vectors of certain diseases. US vector-borne diseases controlled by pesticides have included malaria, plague, equine encephalitis, spotted fever, and endemic typhus.

The record of pesticides against malaria is impressive. According to the Mrak report, some 60 percent or more of the DDT manufactured has been used for this purpose.

Most pesticides are poisons and can be hazards to the user, the environment, and the food consumer. Yet, when used in discreet quantities and directed specifically at crop pests, pesticides have minimal environmental impact.

Mertz³ suggests that all substances are toxic and all substances should be treated on the basis of a deficiency state, considering that they will produce beneficial responses at some dose level. For instance, life would not exist without boron—a fairly potent toxin.

Vitamins, hormones, and trace elements typically follow a response similar to boron. That is, they are essential to life at some low level, but toxic at levels not too much greater.

Selenium is an essential element in animal diets; yet, it is highly toxic and has been considered a carcinogen (although this is now disputed). Some medical scientists now consider selenium to be an anticarcinogen. In fact, it could be both depending upon its natural abundance or the presence of other substances. The dose response of the macronutrient phosphorus is very similar to that of the micronutrient boron except that it is on an expanded scale. It too is toxic at large dose levels. These levels are unlikely to be found in nature and fertilizer is unlikely to be applied at a toxic level, whereas toxic doses with boron do occur in nature and can easily be introduced into the environment. Another example is 2,4-D. Delicate amounts of 2,4-D, normally a lethal plant killer, increase the size and total yield of solids in tomato plants. Small quantities of simazine may increase the quality and yield of peaches.

In our own field experiments, atrazine residues have significantly increased yields of oats and soybeans without change in protein or lipid content.

Pesticides or related compounds have been used as medicines. For example, DDT is a very effective, and safe, anti-barbiturate. One of its metabolites, DDD, has been used successfully in treating adrenal carcinoma. Some researchers indicate that DDT, aldrin, and dieldrin might reduce the incidence of tumors in experimental animals. Several workers have reported anti-tumorigenic effects of DDT.

Sulfonamide type chemicals—sulfa-drugs effective against venereal diseases and respiratory infections—are now being marketed, with some structural modification, as plant growth regulators. Common biochemicals are routinely screened for biological activity as drugs or pesticides. Both have similar chemical and biological properties.

Drugs have been studied largely for their positive effects, while most of the concern with pesticides has been with potential negative effects. Very little attempt has been made to document

¹Adams was invited by the American Society of Agronomy to write a "direct response" on pesticides. President of the society Fred L. Patterson asked him to present such information, as appears here, to the U.S. House of Representatives' Subcommittee Hearings on "the costs and effects of chronic, low-level environmental pollution"... as related to agriculture.

"The society believes," says Patterson, "that this is an excellent avenue for getting the scientific and technical evidence and opinions before the congressional subcommittee where decisions will be made."

Adams, emphasizes, however, that he "... takes the responsibility for his statements in this magazine, and that they do not necessarily represent interpretations or data from the society."

²"Mrak Report," 1969; Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health; U.S. Department of Health, Education and Welfare.

³Mertz, W.; 1969; "Problems in Trace Element Research," Trace Substances in Environmental Health—11; p. 163; University of Missouri, Columbia.

possible indirect positive effects of the pesticides on animal and human health. Without such information, some of the arguments relating to pesticides can neither be fully substantiated nor refuted.

Vitamins and hormones are receiving the same concern regarding potential carcinogenicity, teratogenicity, etc. as pesticides are today. Vitamin A has been

needs in pesticide research is to develop more effective means of educating people in pesticide use and storage, and to seek medical treatment when excessive exposure does occur.

"An 8-year study on effects of pesticides on humans shows no harm done to those with higher-than-average exposure," according to West Virginia

figures on the total number of deaths from accidental poisoning. Thirty-five people lost their lives from pesticide, fertilizer and plant food poisoning. Thirteen of the pesticide, fertilizer and plant food related deaths in 1974 were accidental poisonings of children under the age of five years. This is compared to 370 people who died of alcohol poisoning and 752 from other chemical poisoning not including pesticides. In the pesticide, fertilizer and plant food related deaths, the figures are down from 72 deaths in 1968, even though today we are using more highly toxic insecticides than we were using in 1968 since the cancellation of five relatively safe pesticides, DDT, Aldrin, Dieldrin, Chlordane and Heptachlor."

The organochlorine pesticides are relatively safe in terms of acute human exposure. On the other hand, acute exposure to either organophosphates and carbamate pesticides may lead to cholinesterase inhibition with serious consequences if not properly treated. Herbicides are generally less toxic than insecticides; but there are exceptions.

The Mraz report concluded that:

"The only unequivocal consequence of long-term exposure to persistent pesticides at levels encountered by the general population is the acquisition of residues in tissues and body fluids. No reliable study has revealed a causal association between the presence of these residues and human diseases."

This conclusion was reached in spite of the fact that the report accepted the still vigorously disputed contention that the organochlorine pesticides are carcinogenic.

Because of their ease of decay—or short persistence—residues of organophosphate and carbamate pesticides are likely to be rare in foodstuffs by the time the food is prepared and cooked for eating. At this point, we have already made a decision involving tradeoffs. More potentially hazardous, but less persistent chemicals have been substituted for less hazardous but more persistent chemicals. The wisdom of this political decision is still debated.

Pesticide residues which reach the consumer generally do so by the oral route. Thus, stomach and bowel cancers are of particular interest to agronomic and agricultural scientists. The *Atlas of Cancer Mortalities for U.S. Counties, 1950-1969*, shows no correlation between gastric cancer and agricultural areas. This does not mean that food, or the oral route, is not involved. Areas where

continued on page 14



Many foods consumed daily, such as some of the products shown here, are thought by various scientists to contain naturally occurring substances that can cause cancer.

implicated in breast cancer. Reduced forms of zinc, manganese, and iron are essential in animal diets. But, they have been implicated as cancer promoters. Such elements are present in nearly all living tissues on earth and make carcinogenic substances universally present.

Most of the reported impacts of pesticides on human health are immediate and due to acute exposure. With the banning of the so-called hard pesticides for many uses, these incidences have risen. Such serious illness or death is usually a direct result of improper or inadequate storage or disposal of unwanted chemicals, and inadequate medical treatment.

California has had the most extensive use of pesticides on crops directly eaten by humans. They have reported the greatest incidence of pesticide poisonings, almost all accidental. Over half of these poisonings were skin disorders. About two-thirds of the poisonings occurred with children. Clearly, one of the greatest

University's "Pesticides and Chemicals Program Report" of Sept. 1976:

"Scientists from the Mississippi Agricultural and Forestry Experiment Station made the study—part of the federal Community Studies Program. Federal researchers instructed each state to choose from specific occupationally high-exposure subgroups, including pesticide manufacture, formulation and distribution; farming; agricultural research, and public health. The Mississippi group included farm laborers and pesticide plant workers, and a few aerial applicators.

"Lower exposure groups used as checks included persons with jobs in urban areas, such as teachers, garbage collectors and street maintenance crews. No adverse effects of any kind could be found, and doctors examining the volunteers agreed that no detectable damage to health was evident."

". . . The National Clearing House for Poison Control Centers (HEW), Washington, has just published the latest

Improving an Old Way to Store Onions

THE INFLUENCE of environmental factors on onions and their storability has long been realized by scientists and onion growers. Storage at a cool dry place has always been recommended and major emphasis in research on storage conditions has been given to the storage temperature, while storage humidity was less tested.

A humidity of about 70 percent or higher is characteristic of the environment of onions under most commercial storage. Onion storage can be enhanced by preharvest spray application of a chemical called maleic hydrazide (MH). Since the growth inhibitor's introduction in 1950, MH has been used by commercial growers to prevent sprouting of onions.

Under warm and moist conditions, onions tend to sprout very soon and become unfit for the market. With the proper dosage of MH, however, current storage limits can be extended to 4 to 5 months when onions are kept cool.

Now, University of Minnesota Agricultural Experiment Station plant physiologists Jiwan P. Palta, Jacob Levitt, and Eduard J. Stadelmann have refined the old chemical-free method to maintain the freshness of onions for a longer period than with standard storage. As with MH, the improved method helps to extend the period for onion storage, but increases the length of the storage period. The improved method involves better control of the humidity of the onion's storage environment.

Shoot development is controlled by temperature but not by humidity, as has

been known. With an increase from 2°C to 5°C, the percentage of onions with visible shoots is more than doubled.

Root development, on the other hand, is promoted by high humidity.

"Humidity values lower than 70 percent, however, were never tested before," says Palta.

"Such a low humidity also considerably decreases the shoot growth." When stored for 11 months at a relative humidity of 40 percent or less, only 20 percent of the onions have visible shoot growth. About 70 to 81 percent of the onions stay fresh for marketing and 80 to 97 percent remain good enough for eating."

The usual commercial storage period of onions is about 5 to 6 months.

"Considerable potential exists for the successful long-term storage of onions by using humidity below 40 percent at 1°C to 3°C," says Palta.

"This will, however, require an economical way of lowering the humidity at this lower temperature. Humidity control in the storage facility at present, however, may be more costly than the application of the growth inhibitor in the field."

The University of Minnesota workers hope that the results presented here will serve the purpose of reviving interest in more studies on the environmental method for extended onion storage. It is remarkable that onions may be kept for as long as about a year whereby 85 percent remain marketable, when the humidity remains below 40 percent and the temperature is kept under 5°C. □

Minnesota researchers stored these onions at relative humidities of 5, 40, 75, 90, and 100 percent. Notice the evidence of increased root growth at increased humidity.



gastric cancer incidence is highest are found in northern Minnesota, north central North Dakota, and north central New Mexico. Because these areas are remote from large urban centers, natives are likely to rely more heavily on their own water supplies (often untreated) and home gardens.

According to Barnes⁴ 100 ppm aminotriazole (cranberry scare) fed continuously for 2 years in rat diets produced thyroid cancer in 4 of 26 rats. Cabbages, turnips, peas, beans, strawberries, and milk contain natural, and related, chemicals. Rutabagas naturally contain up to 200 ppm of a potent thyroid cancer inducer (L-5-vinyl-2-thioxazolidine). Astwood claimed that one serving of Swedish turnips may contain 100 times as much thyroid cancer-inducing activity as cranberries badly contaminated with aminotriazole.

Nature abounds with naturally occurring toxic chemicals. The levels that these occur in foodstuffs often exceeds the safety factors established for pesticides. This does not imply that foodstuffs are unsafe, only that natural toxins exist in our food at levels exceeding safety margins required of pesticides. Many of these natural toxins have herbicidal, insecticidal, and fungicidal properties and their chemistry may not differ greatly from the pesticides.

The compound alpha-benzpyrene is perhaps the most potent carcinogen known to man and is commonly found in soil particularly in forest soil humus. Several studies have shown that the occurrence of gastric cancer is related to the occurrence of water-logged acid soils high in humus or receiving excessive amounts of organic manures.

There is an imperfect, but nevertheless striking relationship between the incidence of gastric cancer and the occurrence of acid bogs or the fossil remains of such bogs, such as petroleum shales and brown coals.

Humus has the unusual ability, particularly under water-saturated and acid conditions, to make zinc more available and copper virtually unavailable. Zinc-copper ratios may be critical, as some medical scientists consider zinc a cancer promoter and copper a cancer inhibitor or anti-carcinogen. Some researchers also see a relationship between zinc-copper ratios and arteriosclerosis and heart problems.

Reading the roster of organic chemicals credited with promoting or inducing cancer is like reviewing the

chemistry of soil organic matter or humus. A class of chemicals, polyphenols, often implicated in promoting or inducing cancer forms the building blocks from which soil humus is produced. Polyphenols may account for 50 percent of the dry weight of tea leaves.

Until proven or disproven, a relationship between acid bogs and cancer or soil and cancer can only be speculative. Furthermore, while the presence of potent, naturally occurring carcinogens are known to exist in soils, few, if any, agronomists could accurately speculate whether these compounds could be taken up by plants and translocated in a biologically active form to the edible portions.

It is ironic that we look upon the occurrence of these compounds as a natural oddity and expend virtually no funds or effort in studying them while on the other hand become greatly concerned about the pesticides. Yet the effect of pesticides on cancer induction in our population cannot be statistically determined when compared to these natural carcinogens because of the latter's relatively greater abundance in the environment, widespread distribution, and level of carcinogenicity. Although requests for support for agronomic research have been made to examine the possible influence of soils and crops on the occurrence of cancer, little or no US funding has been allocated for such studies.

As of July, 1975, finite tolerances had to be established for all pesticides or their registration was cancelled. However, there are those who still insist on "zero" or "no residue" tolerances. As previously administered these tolerances were established either on the basis of assumed potential hazard, or on the basis that no residue should be present if the pesticide were used as prescribed on the label. The latter was the usual case for no-residue registrations. Consequently, the application of these tolerances was not consistent and did not have equivalent biological meaning. Furthermore, zero levels of a quantitative unknown cannot be measured or established. Essentially all analytical procedures will measure a finite amount of a given substance in a sample known to contain none of that substance. This is why analytical procedures contain blanks. In pesticide residue analyses, one rarely has the luxury of a sampling blank or control sample. Thus the true analysis must legitimately reported as something less than 1, less than 2, less than 10, etc. These numbers together with units indicate lower limits of detection below which one cannot say with certainty that

the substance is absent or present. This analytical "zero" lowers as analytical procedures become more sophisticated. Unfortunately, many analysts ignore the lower limit of reliability when reporting pesticide residue data.

What is apparent zero today by one analysis may not be "zero" tomorrow by another analysis. We may be able to measure one part in one trillion; but our level of confirmation may only be one part in one billion or one part in one million.

The practice of a "floating zero based upon changing and improving analytical capabilities places great hardship upon the producer. Production processes installed to comply with one "zero" may have to be significantly modified when a subsequent and more sensitive "zero" is established. This cost must be passed to the consumer in the form of more expensive products, less available products, higher taxes, or a combination of all 3. No one should question the necessity for this with a chemical like aflatoxin, a natural toxin which produces cancer in 100 percent of the test animals at 100 ppb. The necessity for such instability is questioned for one like DDT, which produces tumors of debatable malignancy in 20 percent of the test animals at 800 ppm or 8,000 times as much chemical. A zero threshold concept makes no allowance for risk benefit considerations and since it cannot be truly followed in practice is basically dishonest. When the public learns that tolerances for a compound—which were formerly set at a zero, which really was not—are now being lowered to a zero, which really is not, then the credibility of the regulatory agencies, political systems, and scientists is lowered. The truth would be much more palatable.

The Delaney clause to the Food and Drug Act states:

"That no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal . . ."

The Delaney amendment has been broadly interpreted by some to include carcinogens, mutagens, and teratogens. The question of appropriateness of the tests has frequently been debated. For example, tests frequently used for carcinogenicity establish the ability of the pesticide to promote, not induce cancer. The clause does not state that a proven carcinogen shall be deemed a carcinogen at any level nor does it state that a cancer promoter shall be deemed a carcinogen, although it has been

⁴Barnes, J.M.; 1966; "Carcinogenic Hazards From Pesticide Residues," *Residue Review*; Vol. 13, No. 69.

interpreted in that manner. Strict adherence to the wording would have quite the opposite interpretation. Undoubtedly this has been a major factor surrounding the controversy over this amendment.

The Delaney clause becomes vulnerable because if it were applied to natural substances, sometimes essential substances, no foodstuff could be legally sold or consumed. Some of the natural carcinogens are among the most potent known to humans.

Examples of these dangerous, but natural, carcinogens are: aflatoxins produced by the grain-spoilage fungus, *Aspergillus flavus*, and the pyrrolizidine alkaloids produced by some pasture weeds eaten by dairy cows and then excreted in milk. Fungicides and herbicides can control such sources of carcinogens.

Even though no residue of a substance is permitted by law, enforcement agencies must set some lower value as their analytically reliable zero. The author understands that for aflatoxins this is 15 parts per billion. A straight line response curve would tell us that aflatoxin could be "legally" present at a rate which would bring death from cancer to an estimated 150,000 consumers in one million. In comparison, DDT—which has the same detection limit—would produce tumors in an estimated 3 to 4 people in one million at the same level of exposure.

In an attempt to reduce exposure, guidelines such as the Delaney amendment have come into being.

The concept behind the Delaney amendment is sound. No one would advocate permitting a substance in food quantities that would present a threat of cancer. However, its wording is vague and it has been used as a political tool rather than a mechanism for setting standards. Consequently, the Delaney amendment has not been fairly or consistently applied.

The question of a dose response, which is implicit in the way the Delaney clause has been applied, is hotly debated. There are those that contend that there are no safe thresholds. They point out that the threshold concept is a non-linear relationship between dose and response which describes a significant no effect level. Critics of the concept of thresholds maintain that a carcinogen may produce cancer at any dose level and, thus, follows a straight line response.

This concept is theoretical and undoubtedly will stimulate academic debate for some time to come. However, with this logic one must conclude that at

extremely low levels a comparatively weak carcinogen becomes as dangerous as a comparatively strong one.

Regardless of these arguments, detectable cancer—as a result of a specific inducer—must follow a non-linear response curve for at least 3 reasons: multiple variables, test limitations, and substrate deactivation.

With any substance that produces an additive carcinogenic response 2 variables determine the extent of that response: dose and time of exposure. Normal exposures would not be expected to be uniform. From time zero, each exposure would have a different accumulative time. Mathematically this can only be described by a surface, not a straight line. If dose is plotted against incidence, ignoring exposure time and differential response to exposure time then the result must be non-linear. Dose-time response curves can be used to predict statistical probabilities of cancer induction. These predictions should be used in establishing guidelines for "trade-off" decisions, which now must be made arbitrarily and without consistent adherence to scientifically reliable information.

Laboratory rats and mice are often selected for cancer experiments because of their susceptibility to cancer, which therefore becomes a common natural death for these rodents even when not experimentally exposed to synthetic cancer-producing chemicals. As a result it is statistically impossible to demonstrate carcinogenicity in these test animals unless a dose response is shown. Since cancer occurs in the control animals and there is a difference in susceptibility among animals, again, the response must be non-linear. Whether the high susceptibility of rats to cancer gives the test a greater degree of sensitivity or whether the necessity of a dose response makes it less sensitive needs to be objectively answered.

With legitimate carcinogens, the dose effect is quite pronounced. For example, where dimethylnitrosoamine was included in rat diets: rats fed 50 ppm in their diet all developed liver cancer in 364 days; fed 5 ppm all but two rats died of liver cancer within 500 days (a laboratory rat's expected life span is 2 to 2½ years); and fed 2 ppm no rats developed tumors after 525 days. Many medical scientists believe that tumors must become malignant to prove carcinogenicity. Many compounds, such as the organochlorine pesticides, produce tumors when included in the diet of laboratory animals. With these chemicals, these tumors have often been shown either to disappear when withdrawn from the diet, or little dose response has been

noted. Some argue that a non-malignant tumor will become malignant if it persists long enough. Whether anyone has been able to reproducibly induce malignant tumors with the organochlorine pesticides is debated. The Environmental Protection Agency recently accomplished the ban of aldrin and dieldrin by requiring only that tumors be formed when the pesticides are included in the test animal's diet.

In practice it would be impossible to show a straight line dose response curve without artificial administration of a carcinogen, i.e., intravenous injections or administration via a solvent into the alimentary canal in the total absence of any other matter. Portions of organic residues quickly bind to organic or mineral material; most bound residues are unavailable to the consuming animal. Research has shown that when test animals fed foodstuffs containing bound residues were sacrificed, and all matter removed from the alimentary tract, all bound residues remained in the gut or were passed in the fecal matter.

If pesticides were administered orally, binding would be expected to occur to nondigestible fibers in the stomach or intestines. The smaller the dose, the greater the amount of binding that would be expected to occur. Binding or deactivation of pesticides by adsorbents depends upon the chemical nature of pesticide and the adsorbent, the length of time the pesticide is in contact with the adsorbent and the concentration of the pesticide. A considerable portion of pesticide residues occurring in foodstuffs would be expected to be bound.

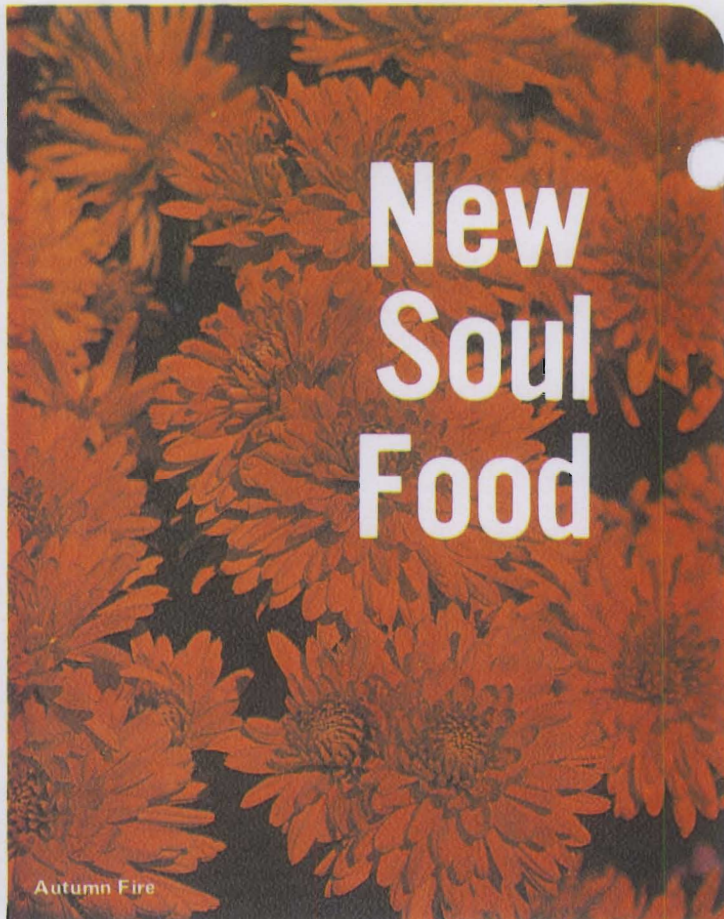
Discussions regarding carcinogenicity of pesticides become further complicated when considering the question of multiple insults, i.e., one carcinogen may be additive to another. There is some evidence that one carcinogen may act as an anticarcinogen in the presence of another carcinogen.

In view of the widespread distribution of naturally occurring carcinogens, the presence or absence of anticarcinogens may be of greater importance in establishing incidences of cancer. This again may be a matter of dose.

The complexity of factors that must be considered in evaluating the potential hazard of a given chemical or foodstuff is great. However, rarely will we be presented with clear-cut alternatives. We must establish finite tolerances for substances that we wish to avoid. Intelligent choices cannot be made without some measurable standard on which to base that choice and some evaluation of risk-benefit of all alternatives. □



Golden Star



Autumn Fire

New Soul Food

MANY PEOPLE WANT pretty plants and experiment station researchers develop new and hardy ones.

"An ecology spurt started about 1973 and has been growing ever since," says Richard E. Widmer, of the University of Minnesota's Department of Horticultural Science and Landscape Architecture.

"This new reawakening to nature began with young people," he says, "and will last."

He says potted plants are in demand because they often last longer than fresh-cut flowers.

"Flowers are food for the soul," says the UM horticulturist. "This soul food nourishes mental health and helps to reduce tensions. Growing flowers also gives opportunities for physical exercise. Plants are therapy in homes."

One of the early gifts a child can give is a flower, if only a dandelion.

"In Detroit and Harlem, kids protect trees and other plants," says Widmer.

One German study, he says, showed that plants in the work area decrease absenteeism.

"People have a craving for nature," says the Minnesota scientist.

The horticulturist cites the increase in number of greenhouses, and increased production of green plants and bedding plants—for the garden—as examples of the ecology spurt.

"Part of the ecology spurt may be because we were at war so long and now we have a different outlook," he says.

Five times as many students are now enrolled in horticulture as there were in 1971, he points out.

"Such growth is also a national trend," says Widmer. "And the average quality of the horticultural student is up."

The Minnesota Agricultural Experiment Station announces 2 of Widmer's and UM horticulturist Peter D. Ascher's new chrysanthemums for 1977:

Golden Star is a single flowered delicate-looking variety with surprising frost and weather resistant flowers. It blooms from August to November (or killing frost). Notice the spoon-petals.

It has willowy-stiff stems, medium-green leaves, and grows in full sun in mounds about 13 inches high and 20 inches wide.

Another new variety to be released for the 1977 gardening season is *Autumn Fire*, announce Widmer and Ascher. It is excellent for spring blooms in indoor pots, outdoor borders, and as an outdoor fresh supply of flowers from mid-September until a killing frost.

For more information about these chrysanthemums, or about 24 other popular varieties of chrysanthemums introduced by the experiment station, ask the UM Bulletin Office for Miscellaneous Report 140 of the University of Minnesota Agricultural Experiment Station, St. Paul. □

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