

FRONTIERS of PLANT SCIENCE

FALL 1977

State Insect
See Page 6



THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION NEW HAVEN

Net photosynthesis can be doubled by inhibiting glycolate formation

By David J. Oliver

In recent years it has become obvious that in many plant species about 50% of the carbohydrates formed by photosynthesis are oxidized back to CO₂ and lost to the air before they can be used or stored by the plant. This wasteful process is called photorespiration. Photorespiration decreases net photosynthesis and as a result decreases crop yields.

Unlike normal respiration in the mitochondria deep within cells where the energy of carbohydrates is available for cellular growth and development, the energy released by photorespiration is lost as heat. Photorespiration occurs through a sequence of biochemical reactions known as the glycolate pathway of carbohydrate metabolism (Fig. 1). The amount of CO₂ photosynthetically fixed into carbohydrates available to the plant is

The decreased rate of glycolate synthesis results in a 60% decrease in the rate of photorespiration and a doubling of the net rate of photosynthesis.

equal to the total CO₂ fixed less CO₂ lost by photorespiration.

Loss by photorespiration does not occur in all crop species. Species without photorespiration, such as corn, sorghum, and sugarcane can be compared with those which show photorespiration, such as soybean, wheat, potato, and rice to illustrate the reduction in crop yield caused by photorespiration. In 1975 the average grain yield per acre of corn in this country was 86 bushels, while soybean yielded only 28 bushels.

Israel Zelitch, Mary Berlyn, and I are directing most of our research toward making the second group of plants more efficient in converting sunlight and CO₂ into carbohydrate by slowing photorespiration. To understand my approach, one should know how metabolic pathways work in cells.

In most biochemical pathways the rate at which compounds move through the reactions is tightly regulated. This assures that the end products are available when needed and only at the desired concentrations, and that no intermediate products accumulate to toxic levels. This is often accomplished by a process known as feedback inhibition. When an end product accumulates beyond a desirable level, it binds to an enzyme near the beginning of the pathway, slowing or stopping that enzyme's activity. With the beginning blocked, raw materials used in a particular pathway are used for other cellular functions.

When I started working at the Experiment Station, Dr. Zelitch and I had several discussions about the glycolate pathway. Most scientists assumed that glycolate was produced because of an unalterable reaction between the enzymes that fix CO₂ and O₂ in the atmosphere; therefore they felt that no control functions would exist. However, we were not convinced. To find feedback inhibitors that would slow photorespiration we first sought compounds which decreased the rate of synthesis of the first known product in the pathway, glycolate.

The laboratory technique I used is based on an observation Dr. Zelitch made several years ago that certain sulfonic acids cause glycolate to accumulate in leaf tissue because they block the enzyme glycolate oxidase

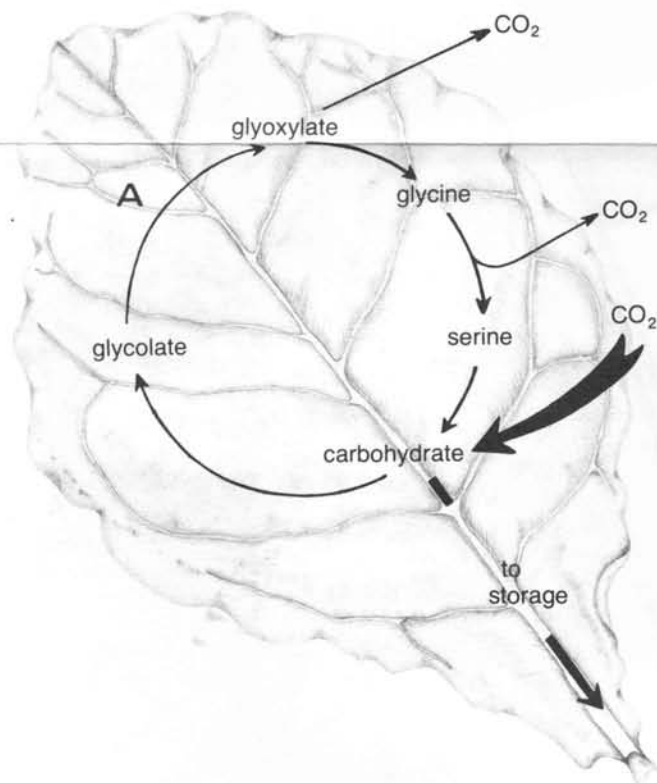


Fig. 1. The glycolate pathway of carbohydrate metabolism. Carbohydrates formed by photosynthesis from CO₂ in the air can be lost through the glycolate pathway, which converts carbohydrates back to CO₂ by the oxidation of glyoxylate and glycine.

(reaction A in Figure 1). The rate of glycolate synthesis can be calculated from the amount that accumulates in the presence of these sulfonic acids. To test the effect of a compound on glycolate synthesis 1.6 cm discs punched from a tobacco leaf are floated on a solution of the chemical. After 1 hour the liquid is replaced with a sulfonic acid solution for 3 minutes. The discs are killed by boiling in 20% ethanol. The discs are homogenized to extract the cell contents, the glycolate is purified by column chromatography, and the amount of accumulated glycolate is measured using a colorimetric assay.

To prove that inhibitions are not artifacts of the assay system it is important to show that these compounds, in addition to inhibiting glycolate synthesis, would inhibit photorespiration and as a result stimulate net photosynthesis. I measure photosynthesis by stringing six leaf discs together with a cotton thread and placing them in the bottom of a specially designed flask. I then carefully add a water solution of the compound to the flask until the discs just float.

I determine the rate at which the leaf discs photosynthetically fix CO₂ by passing a stream of air labelled with radioactive ¹⁴CO₂ over the surface. After a 5-minute exposure I grab the thread with a tweezer and plunge the discs into boiling ethanol. The rate of CO₂ fixation is calculated by measuring the radioactivity in the discs.

Unfortunately, I had no way of telling which compounds act as feedback inhibitors. The only approach that seemed valid was to test a number of compounds which are involved in the glycolate pathway, the CO₂ fixation cycle, or are generally important in cellular metabolism. After searching the shelves and testing three or four dozen such chemicals, I found four compounds that seemed to consistently inhibit glycolate synthesis and thus inhibit photorespiration. Of the 14 amino acids tested, I found only glutamate and aspartate effective. None of the carbon reduction cycle intermediates and only glyoxylate from the glycolate pathway (Fig. 1) inhibited photorespiration. Phosphoenolpyruvate also proved effective. I studied glutamate and glyoxylate in the greatest detail.

When I floated tobacco leaf discs on glutamate for 1 hour, their rate of glycolate synthesis was inhibited about 40%. This inhibition resulted in a 40-60% reduction in the rate of photorespiration. This decrease in photorespiration increases net photosynthesis by 20-25%. This increase is not readily reversed when the glutamate concentration returns to normal.

Glyoxylate has even more dramatic effects on photorespiration. A solution of glyoxylate inhibited the rate of glycolate synthesis over 90% in leaf discs. This inhibition, unlike that seen with glutamate, is readily reversed when the excess glyoxylate is "washed out" of the tissue. The decreased rate of glycolate synthesis results in a 60% decrease in the rate of photorespiration and a doubling of the net rate of photosynthesis. We are

now designing experiments to show the exact biochemical mechanism by which glyoxylate and glutamate inhibit glycolate synthesis.

My next step, in association with Dr. Berlyn, a geneticist in our laboratory, was to select for mutant plants in which the normal mechanisms for controlling the glutamate or glyoxylate levels were altered in such a way that they would have higher cellular concentrations of these compounds. To accomplish this, we grew cells of a crop plant in a culture like microbes.

Since each plant cell contains all of the genetic information (DNA) needed to form an entire plant, each cell can be treated as an individual plant, and the equivalent of millions of plants can be grown in a single petri dish. Cells can be mutated (usually by ultraviolet light) and those that are resistant to some normally toxic substance can be identified by growing them on a media that contains the compound. The resistant cells grow into a clump called a callus while normal cells die. Under the proper conditions a callus will form shoots and then roots. This plant will be identical to the plant that was used as a source for the cells except that it will contain the mutation selected.

Dr. Zelitch, while searching the scientific literature, found a compound, α -methylaspartate, which is structurally similar to both glutamate and aspartate and therefore interferes with their metabolism. Dr. Berlyn has shown that this compound is toxic to isolated plant cells grown in tissue culture.

We have now selected several clones of tobacco cells that are resistant to α -methylaspartate on the assumption that some may escape the toxic effects by having greater than normal glutamate or aspartate concentrations. Increasing the concentration of either of these amino acids in the tissue may have the same effect as increasing their concentration in leaf discs. If this holds true, plants derived from some of these clones may be expected to have decreased glycolate synthesis and photorespiration and as a result increased net photosynthesis, and, most importantly, crop yield.

Suggested Reading

- Oliver, D.J. and Zelitch, I. *Metabolic Regulation of Glycolate Synthesis, Photorespiration, and Net Photosynthesis in Tobacco by L-Glutamate*. *Plant Physiology* 59, 688-694 (1977)
- Oliver, D.J. and Zelitch, I. *Increasing Photosynthesis by Inhibiting Photorespiration with Glyoxylate*. *Science* 196, 1450-1451 (1977)
- Zelitch, I. *Photosynthesis, Photorespiration, and Plant Productivity*. Academic Press, New York (1971)
- Zelitch, I., Oliver, D.J., and Berlyn, M.B. *Increasing Photosynthetic Carbon Dioxide Fixation by the Biochemical and Genetic Regulation of Photorespiration*. In *Biological Solar Energy Conversion* (ed. Mitsui, A., Miyachi, S., San Pietro, A., and Tamura, S.) Academic Press, pp. 231-242 (1977)
- Zelitch, I. *Improving the Efficiency of Photosynthesis*. *Science* 188, 626-633 (1975)



Fig. 1. A red pine plantation in the final stage of attack by the red pine scale. The branches of infested trees gradually succumb starting at the bottom and moving toward the top.

Is the red pine scale adapting to colder temperatures?

By George R. Stephens

During the past 30 years the sight of gaunt, lifeless red pine has become increasingly more common in southwestern and central Connecticut. The cause is a tiny insect known as the red pine scale, *Matsucoccus resinosa* B. & G. Red pine, found naturally in a few small areas of northwestern Litchfield County, had promising characteristics and was widely planted (about 30,000 acres) during reforestation earlier in this century. The scale, whose only other known hosts are three imported pines, was first noticed in 1946 at Easton and is presently found only in Connecticut, southeastern New York, and northeastern New Jersey. Thus, we have an unusual situation of a tree species growing out of its natural range and an insect of obscure origin, which produces disastrous consequences.

The scale has spread from Easton, leaving devastated red pine plantations in its wake. Because the scale spends most of its life resting or feeding beneath protective bark flakes, control with insecticides is difficult on ornamental trees and prohibitively costly in forest plantations. Effective predators or parasites are lacking.

We have an unusual situation of a tree species growing out of its natural range and an insect of obscure origin, which produces disastrous consequences.

Since biological control appears ineffective and chemical control is difficult, is there any other control? In observing the spread of the scale I noticed marked parallelism of the current western and northern boundary of the infestation with isotherms of mean annual temperature. Mean annual temperature is a melding of the highs and lows throughout the year, while isotherms are imaginary lines connecting all locations having the same temperature. Just as the relative closeness of contour lines on a topographic map indicates the steepness of slope, so does the proximity of isotherms portray how temperature or local climate changes. In general, the scale has spread less rapidly across isotherms from a warmer to a cooler environment than it has parallel to the isotherms where there is little change

in temperature. By itself this observation is inconclusive, but it suggests that temperature does affect the rate of spread of the scale. Indeed, 20 years ago a scientist in Yonkers, New York reported that a single 4-hour exposure to -10°F killed 95 percent of overwintering scales. After a second exposure nearly all died. However, -10°F is a rare climatic event throughout most of Connecticut. The likelihood of two or more occurrences of -10°F or below in a single winter is practically zero along the shoreline, rises to 1 in 15 years in the Connecticut Valley; only in the "icebox" of northwestern Litchfield County does -10°F occur with any regularity, about 3 years in 4 or 5. Falls of temperatures to 0° or 5°F are, of course, much more common.

I set about to determine the effect of cold on the survival of overwintering scales. Potted red pine seedlings were infested with scales in late summer, allowed to remain outdoors until January, and then were subjected to cold treatments in a special freezer. After treatment the trees were maintained above freezing. Six to 8 weeks later, as surviving scales developed into the next growth stage, the proportion of dead scales was determined.

In January 1976 one, two or five 4-hour exposures to 0°F increased scale mortality compared to the less severe outdoor temperatures. Mortality of scale was relatively low, 18 to 25 percent on untreated trees and 18 to 75 percent on the chilled ones. Mortality was generally greatest on the 1- and 2-year-old stems of treated trees; unfortunately, on the 3- and 4-year-old stems where the scale population usually flourishes, it was not different from mortality on untreated trees. Chilling late in January caused greater scale mortality than chilling early in January, suggesting that even the less severe outdoor temperature was exacting a cumulative toll on the scale.

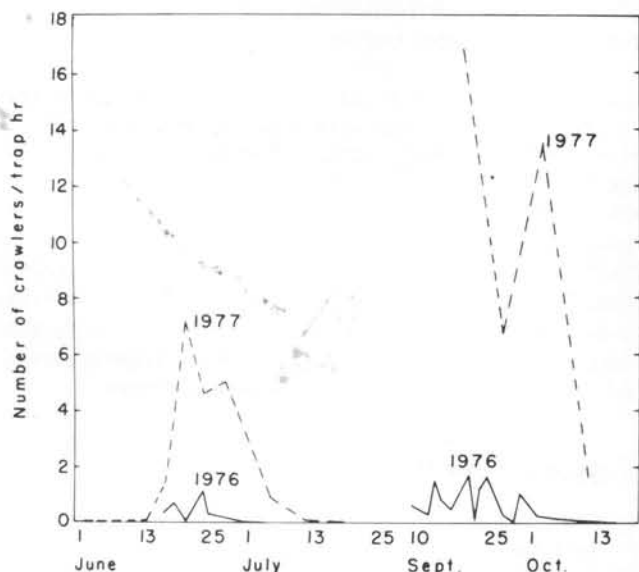


Fig. 2. The increase in red pine scale crawlers trapped beneath an infested plantation in Killingworth. Despite a cold winter in 1976-77 the population has continued to flourish in 1977.

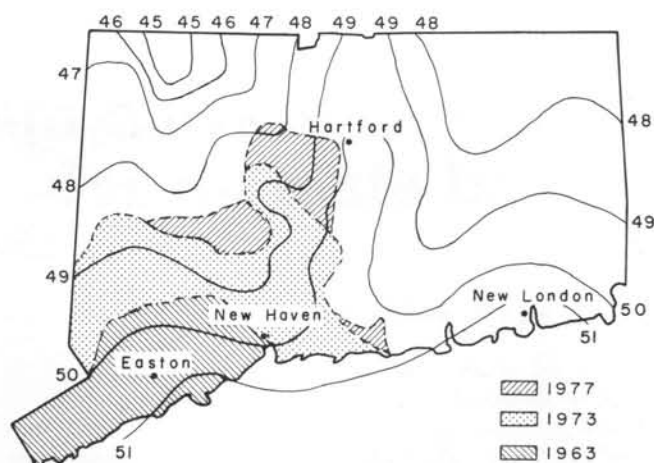


Fig. 3. The relation of isotherms of mean annual temperature ($^{\circ}\text{F}$) to the spread of the red pine scale. The scale has spread more rapidly from Easton in areas of like temperatures than from warmer to cooler areas.

The experiment was repeated in January 1977 with exposures to -10°F . Scale mortality in untreated trees was again low, 2 to 12 percent. In nearly every instance one, two or five 4-hour exposures to -10°F increased mortality, with the greatest mortality occurring on 1-year-old stems as before. However, the 12 to 40 percent mortality observed on chilled trees is far from the 95 percent under similar conditions reported 20 years ago.

To explore the cumulative effect of cold temperature I exposed seedlings to constant temperatures of 5, 0, -10 , and -20°F for intervals of 1 to 80 hours. Again, mortality was greatest on the youngest stems and, in general, the longer the exposure, the higher the mortality. However, even -20°F failed to kill all of the scales, particularly on the 3-year-old stems. This marked difference in response to cold over 20 years suggests selection for a more cold-hardy strain. Experiments to test differences in cold hardiness between scales from the warmer shoreline area and the colder advancing edge of the infestation are planned for January 1978.

What may we conclude? Climatically most, if not all, of Connecticut lies within the potential range of the red pine scale. Further, cold temperature may retard, but will not prevent spread of the scale. Perhaps we can rationalize our eventual loss of the red pine by remembering that most of our red pine is beyond its natural range. But more disturbing is the suggestion that during the past 20 years a hardier scale has evolved. If true, this insect poses a threat to red pine even within its natural range further north and west.

Suggested Reading

- McClure, M.S., 1976. Colonization and Establishment of the Red Pine Scale, *Matsucoccus resinosae* B. & G. (Homoptera: Margarodidae), in a Connecticut Plantation. *Environ. Entomol.* 5:943-7.
- Anderson, J.F., et al. The Red Pine Scale in North America, A Report to the 1975 Eastern Plant Board. The Conn. Agric. Expt. Sta., New Haven. Bull. 765. 6 p.

European praying mantis named state insect

By John F. Anderson

*The 4th-grade class of the Center Road School in Vernon believed that it was time for Connecticut to honor an insect along with the State flower, bird, animal, and tree. They also wanted to understand more fully the operation and responsiveness of government. Thus, they met with their Representative, Chester W. Morgan, who, along with Rep. Robert M. Walsh of Coventry, introduced a bill designating a praying mantis as the State insect. Although confronted with some opposition, the bill ultimately passed. Gov. Ella T. Grasso traveled to the school to sign the bill into law on May 31, 1977. The European praying mantis, *Mantis religiosa* Linnaeus, officially became the State insect on Oct. 1, 1977.*

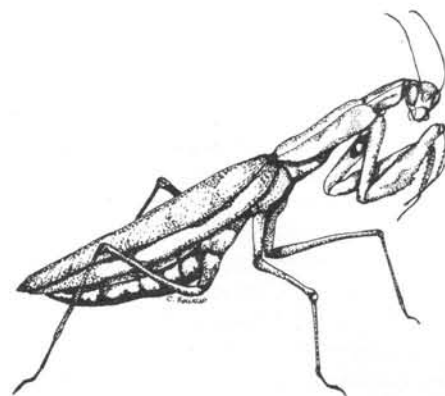
Mantids have long fascinated humanity and they continue to do so in Connecticut. Mantids were well known to the ancient Greeks, who gave them a name meaning "prophet." A number of superstitions have arisen because of their unique stance, which is suggestive of prayer. One prophesy says: "When a mantis alights on your hand, you are about to make the acquaintance of a distinguished person; if it alights on your head, a great honor will shortly be conferred upon you." After the Governor's visit, the children who spearheaded the drive to name the state insect may rightfully argue that there may be more to this belief than superstition.

Praying mantids were first found in Connecticut in 1902 when egg masses of the Chinese mantis were collected in New Haven on a plant imported from Japan. The following year a helping hand was extended by a Station entomologist who distributed additional Chinese mantis egg masses in Connecticut. The insect survived and thrives today in the state.

The European praying mantis occurs naturally in northern Africa, southern Europe and temperate Asia. Egg masses attached to nursery stock were introduced accidentally into Rochester, New York, in the 1890s. The eggs hatched and the mantis became established in North America.

The Experiment Station attempted to establish the European praying mantis here in 1903 but the eggs did not hatch. It wasn't until 1951 that the first European mantids were collected in Connecticut. Twenty-six years later this mantis was to become the State insect. No native North American mantids reside here. The Chinese mantis is the larger of the two and normally reaches a length of 3½ inches. The green or brown European mantis averages 2-2½ inches in length.

Mantids have but one generation per year in Connecticut. They pass the winter in the egg stage. The



young mantids known as nymphs hatch in late May or in June and mature in 2-3 months after molting several times. Adults may be found from late August into October; all adults die from cold weather, predators, or other causes in the fall.

From the time of hatching, mantids feed entirely on insects or other related invertebrates. Aphids and small flies are favorite foods of small mantids. Large nymphs and adults feed on flies, grasshoppers, butterflies, moths, bees, and other medium- to large-sized insects. They usually wait motionless or sway back and forth on leaves or branches until their prey comes within reach. Occasionally they stalk an unwitting insect. Female mantids often eat the heads of their male partners during mating and devour the remaining part later.

Mantids are symbolic of predatory insects useful in biological and natural control. Unfortunately, they are as apt to feed on beneficial as on noxious insects, including their own kind. Overall, they are considered to be beneficial. So voracious are they that one wonders if they might better have been named "preying" instead of "praying" mantids.

Large mantids collected in late summer are relatively easy to maintain in a glass jar covered with screening. Sticks or a small houseplant will provide a suitable stand. Sand on the bottom and blotting paper on one of the sides will provide additional walking and clinging surfaces. Water should be sprinkled on the screen or given to the mantis with a medicine dropper daily. Houseflies, grasshoppers, or other live insects may be fed to the mantis through a small hole in the screen which should be plugged with cotton. Young mantids must be kept in a jar where the relative humidity is 50-70% and require a ready supply of small insects. Cannibalism is not a problem with young mantids unless they are they are overcrowded or underfed. Larger nymphs and adults should be kept separate. Adults may be kept alive for a month or more.

Suggested Reading

- Gurney, A.B. 1951. Praying mantids of the United States: Native and introduced. *Smithsonian Report for 1950*, pp. 339-363.
- Gurney, A.B. 1972. Praying mantids: Exotic insects in our gardens. *Frontiers* 36(5): 14-17.
- Slingerland, M.V. 1900. The common European praying mantis, a new beneficial insect in America. *Cornell Univ. Agr. Expt. Sta. Bull.* 185: 35-47.

Relating the date on carton to freshness of milk

By Lester Hankin

Since 1974, Connecticut law has required that pasteurized milk for retail sale be marked with the last date that it may be sold or offered for sale. This "code date" is set by the processor, not by regulation. Thus, an appraisal of code dates seemed to be of value to dairies and consumers.

George Stephens of the Station staff, Walter Dillman of the Dairy Division of the Department of Agriculture, and I set out to determine the real meaning of the code date by sampling milk and judging its keeping quality in relation to the code date. The tests were an extension of our research on the shelf life or keeping quality of dairy products and other perishable foods.

Each dairy selling milk in Connecticut told us the length of its "code period." The code period is the number of days between the time the milk is bottled and the code date. We found that individual dairies have different code periods; the shortest was 7 days and the longest was 15 days. Because consumers know only the code date marked on the container and not the code period, they have no way of accurately determining the age of milk at the time of purchase. For example, consider two bottles of milk, each showing 2 days remaining to the code date: the milk from a dairy using a 7-day code period would be five-days-old, while milk from a dairy that uses a 15-day code period would be 13-days-old.

In a significant number of cases we found that the code period, and therefore the code date, bore no relation to the number of days it took a sample of milk to develop an unacceptable flavor or odor. The determination of acceptable flavor, and thus keeping quality, was made by experienced milk tasters.

For milk purchased at retail outlets during 1975 we found that processors overestimated their code periods by an average of 2 days. That is, the average sample remained acceptable in flavor only to 2 days before the code period expired. However, for individual dairies the overestimate varied from 0 to 8 days. All but one-

In a significant number of cases we found that the code period, and therefore the code date, bore no relation to the number of days it took a sample of milk to develop an unacceptable flavor or odor.

half of one percent of the samples were within their code period, yet data in Table 1 show that 15 percent of the 864 samples had an unacceptable flavor before their code date was reached.

Proper refrigeration is essential to maintaining quality of milk. In general, the lower the storage temperature, the longer the shelf life, since colder temperatures hinder growth of microorganisms that can produce a wide variety of off-flavors and odors.

It should be pointed out that milk processors lose control of their product once it reaches stores. Thus, we studied the temperature of the milk samples at the time of collection. Table 2 shows that 95 percent of the samples were stored at a satisfactory temperature (45°F or below). However, there is no way of knowing whether this temperature was maintained prior to reaching the point of sale. The fact that 14.2 percent of the unacceptable samples were at 45°F or less when collected suggests that these samples may not have been under proper refrigeration all the time.

Table 2. Temperature of milk collected at stores.

Temperature	% of samples
less than 40° F	41.2
40 to 45° F	53.4
more than 45° F	5.4

We conducted further experiments to estimate how many unacceptable samples could be attributed to the processor and how many could be attributed to less than optimal refrigeration at the store. To test this we collected milk at the dairy at bottling. Each of three bottles of milk was stored at a different temperature and tasted regularly to determine how long the flavor remained acceptable. The results are shown in Table 3. Milk stored at 35°F remained acceptable for an average of 17.5 days. This was much longer than the assigned code period, which averaged 10.8 days. The number of days that the milk remained acceptable declined with increasing storage temperature. At 50°F, only 4 percent

Table 1. Condition of 864 samples of milk collected at stores during 1975.

<u>Unacceptable flavor</u>	
within code	14.7%
past code	0.5%
<u>Acceptable flavor</u>	
within code	84.8%
past code	0

Paul E. Waggoner
Director

PUBLICATION
PENALTY FOR PRIVATE USE, \$300

POSTAGE PAID
U.S. DEPARTMENT OF
AGRICULTURE
AGR 101

THIRD CLASS
BULK RATE



Freshness of Milk (Continued)

of the samples were of acceptable quality on the code date. But even the 9 percent unacceptable rate at 35°F is probably inflated because some of the off-flavors are not microbial in origin. Still, the data prove that the lower the storage temperature, the longer the shelf life of the milk.

We interpret these results to mean that stores must bear a portion of the responsibility for the quality of the milk. Shelf life is probably reduced even more by storage in the home refrigerator, since temperatures likely average between 45 and 50°F, and the milk is removed intermittently for use.

After determining that the code dates are not necessarily an accurate reflection of keeping quality of milk, we compared the keeping quality at 35 and 42°F with keeping quality at 50°F and found good correlation. Thus, processors could store test samples of their milk at 50°F and predict how long their milk will remain acceptable at lower temperatures. Using this test they could obtain an accurate assessment of the useful shelf life of their product in half the time that would be required at 35°F storage. They could also use this information to predict the shortened shelf life under storage conditions encountered between the time the milk leaves the processing plant and is purchased by the consumer.

Further, we have tried to correlate the standard plate count, a test now in use to determine total number of bacteria, with tests that we have devised to differentiate between groups of bacteria that are able to degrade the various constituents of milk such as protein and fat. It is degradation of these materials that gives rise to compounds that can create off-flavor or odor in milk. Little relationship was found between the keeping quality of

milk and the total number of bacteria in the sample. However, with our tests we were able to account for a reasonable proportion of the observed variability in keeping quality.

Table 3. Shelf life of milk stored at different temperatures.

Temperature	av. no. days remaining acceptable	% of samples unacceptable before code date
35°F	17.5	9
42°F	12.1	43
50°F	7.0	96

We are continuing our research to find rapid tests to predict keeping quality, perhaps even one that can be carried out the day milk is bottled. One aspect is to be able to detect groups of microorganisms that can grow under refrigeration temperatures and assess their role in producing off-flavors and odors. Hopefully this information can help dairies ensure the freshness of milk sold to consumers.

Suggested Reading

- Hankin, L., W.F. Dillman, and G.R. Stephens. 1977. Relation of code dates to quality of milk sold in retail markets. *J. Food Protection* 40:116-119.
- Hankin, L., W.F. Dillman, and G.R. Stephens. 1977. Keeping Quality of Pasteurized Milk for Retail Sale Related to Code Date, Storage Temperature, and Microbial Counts. *J. Food Protection*, in press.
- Hankin, L., G.R. Stephens, and W.F. Dillman. 1975. Quality Control Significance of Special Media for Enumeration of Microbial Groups in Cottage-type Cheese. *J. Milk and Food Technol.* 38:738-744.

Frontiers of Plant Science

The Connecticut Agricultural Experiment Station.

Vol. 30 No. 1 Fall, 1977

published in May and November, is a report on research of

Available to Connecticut citizens upon request.

Paul Gough, *Editor*

ISSN 0016-2167