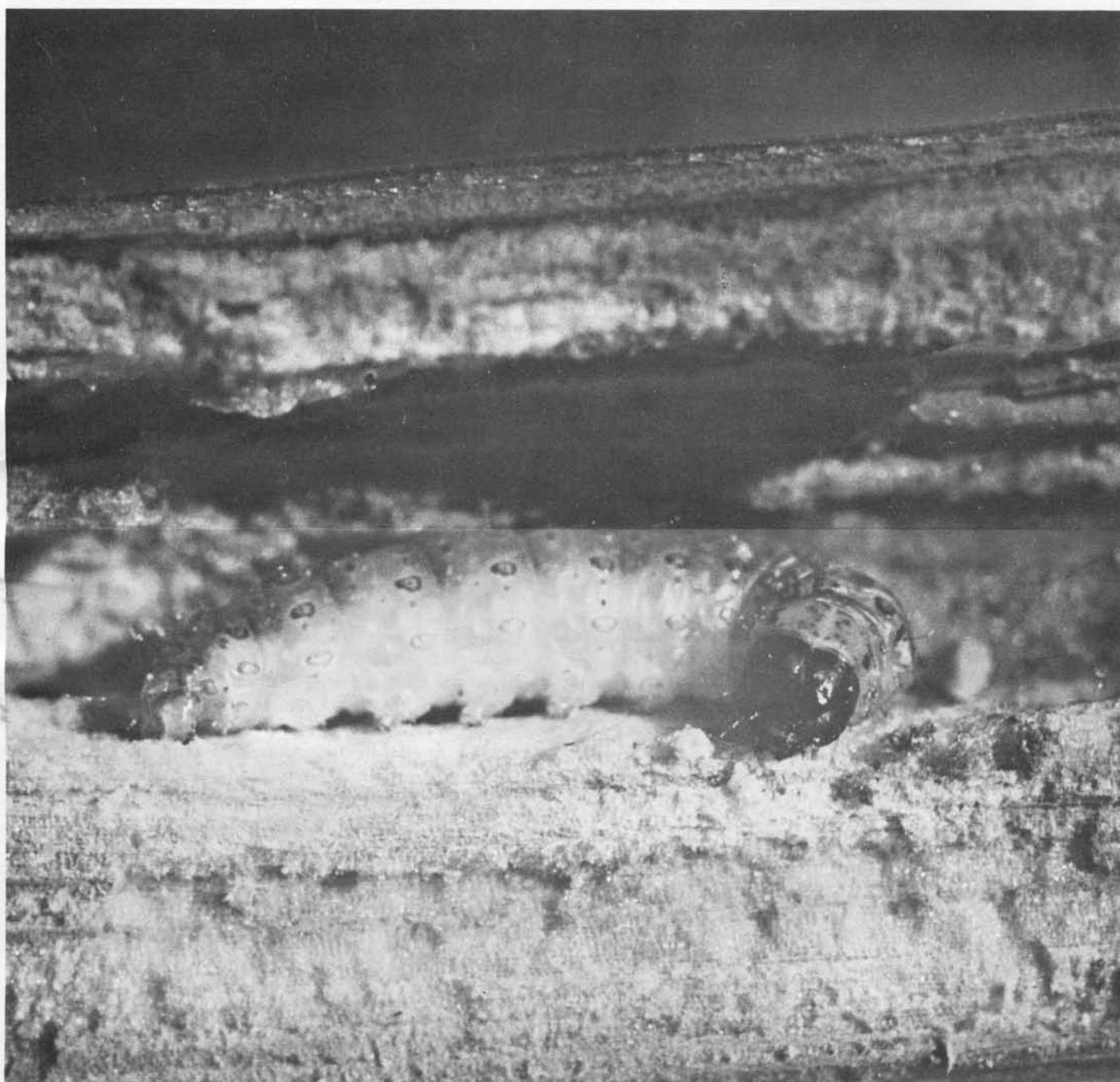# FRONTIERS
of PLANT SCIENCE

FALL 1980

European corn borer

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION   NEW HAVEN

The European corn borer

A generation of the European corn borer develops through four stages: egg, larva or caterpillar (commonly called borer), pupa or resting stage, and adult moth. In Connecticut there are two generations a year. The corn borer passes the winter months of September-April as a full-grown caterpillar within old corn stalks. During late May, the borers transform into pupae. In early June, the first adult moths, which are pale yellow to light brown in color and 1¼″ long, begin to emerge. Adult moths are active only at night and live from a few days to several weeks. The adult females lay an average of 400 eggs. The eggs are usually laid on the undersides of leaves in scale-like clusters of 10 to 25. During warm weather, larvae hatch in less than a week and feed briefly on foliage. Then they tunnel extensively into stalks, tassels and ears where they continue to feed. They pupate in July, and in early August the adults of the second generation begin to emerge. The peak egg-laying occurs in mid-August. Larvae that hatch from these eggs mature in September and hibernate.

Studying microbial and insect enemies of the European corn borer in Connecticut

By Theodore G. Andreadis

One of the pleasures of summertime in Connecticut is abundant and fresh ears of sweet corn for the dinner table. But, if caterpillars of the European corn borer are found in the ears, pleasure quickly turns to distaste.

The European corn borer, *Ostrinia nubilalis*, has established itself as one of the most destructive and threatening pests of sweet corn in Connecticut. Left uncontrolled, it can cause great economic losses for vegetable growers who must consistently meet the public demand for ears without borers.

The European corn borer, a pest of foreign origin, was first found near Boston in 1917. With no natural enemies to check its expansion, the corn borer quickly spread throughout the eastern and midwestern states.

In 1919, the U.S. Department of Agriculture began a program of releasing in this country a number of corn borer parasites from Europe and the Orient to help regulate the populations of borers. Unfortunately, as effective chemical insecticides came into use, this approach was abandoned. Now, as both home and commercial growers are faced with the problems of insecticide resistance and restrictions on the use of many chemicals, there is renewed interest in natural controls.

With this in mind, I am developing an integrated pest management program to maintain the populations of corn borers below economically harmful levels. Most of my research has been learning how well these imported parasites have survived the ravages of New England winters and determining what impact they are having on populations of corn borers. I have collected thousands of corn borer larvae all over the state and examined them for parasites. Of the 21 exotic parasites that were imported and released in New England, I have recovered two wasps, *Macrocentrus grandii* and *Eriborus terebrans*, which have become established in our state (Table 1).

M. grandii (Fig. 1), which was originally imported from France in 1926, has been found in all areas of the state that we have surveyed. It appears to be playing a major role in the natural suppression of the corn borer, parasitizing up to 20% of the borers. The wasp is prolific, producing 20 to 30 progeny from a single egg deposited in a corn borer host. In addition, its life cycle is well synchronized with the life cycle of the corn borer; the wasp spends the winter as a juvenile larva within corn borer hosts and emerges in the spring to attack more borers.

Unfortunately, the other imported wasp, *E. terebrans*, does not appear to be prospering. From 1928-1935 it was released in East Hartford, Haddam,

Table 1. Natural enemies of the European corn borer.

|	Type	Notes
Insects		
Macrocentrus grandii	wasp	imported, well synchronized with host; high rate of parasitism (7-20%)
Eriborus terebrans	wasp	imported, very low rate of parasitism
Aplomya caesar	fly	native, uses borer as an alternate host
Lixophaga sp.	fly	native, uses borer as an alternate host
Microbes		
Nosema pyrausta	protozoan	causes chronic, debilitating disease; very high rate of infection (21-62%)
Beauveria bassiana	fungus	highly pathogenic but require moderate temperatures and high humidity to cause disease
Metarrhizium anisopliae	fungus	highly pathogenic but require moderate temperatures and high humidity to cause disease
Cephalosporium sp.	fungus	moderately pathogenic
Bacillus cereus	bacterium	cause disease when borer is weakened or under stress
Closteridium sp.	bacterium	cause disease when borer is weakened or under stress
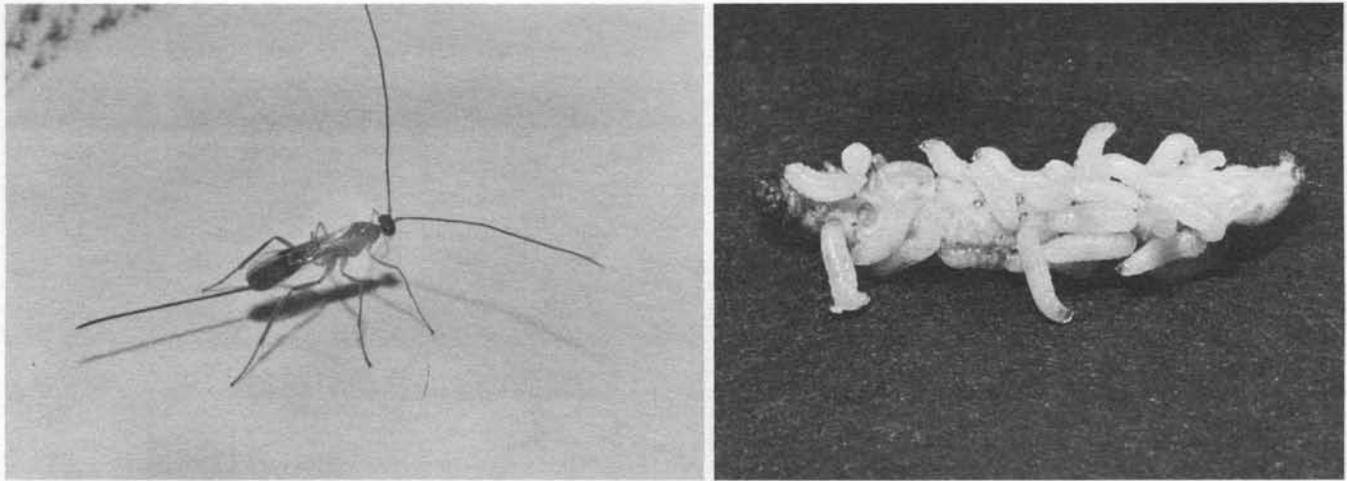
Fig. 1. *Macrocentrus grandii:* left, adult female; right, larvae emerging from the corn borer host.

Milford, and East Lyme. I have only recovered this parasite in Windsor, where it parasitizes less than 1% of the corn borers. Its limited distribution and low rate of parasitism indicate that it exerts little control over corn borer populations.

During this study I have found two other insect parasites. Both are native flies that appear to be using the corn borer as an alternate host. These flies, which resemble a common house fly, are *Aplomya caesar* and *Lixophaga* sp. Although I find them throughout the state, these flies infest less than 1% of the caterpillars; therefore, they also appear to have little impact on corn borer populations.

In addition to insects that attack the borer I have investigated naturally-occurring microbial pathogens as potential control agents. The most promising of these is a small, single-celled protozoan called *Nosema pyrausta* (Fig. 2). This microorganism, which can infect up to 60% of the population of corn borers, is the most important natural control of the corn borer in the state. It produces a chronic, debilitative disease that increases the susceptibility of borer larvae to the stresses of winter and reduces longevity of adults and production of eggs. Over time, these effects upon longevity and fecundity decrease populations. *Nosema* is easily spread because it infects the ovaries of the female moth of the
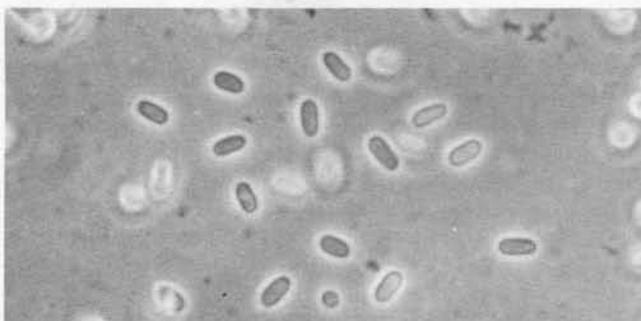
borer and is transmitted in the eggs to subsequent generations.

Unfortunately, I have found that the parasitic wasp, *M. grandii*, is also highly susceptible to *Nosema*. *M. grandii* becomes infected and dies if it parasitizes an infected borer. My surveys show that when the rate of infection of corn borer with *Nosema* is high, parasitism of borers by *M. grandii* is low, indicating that *Nosema* significantly limits or prevents the establishment of large populations of *M. grandii*. This observation may explain why the rate of parasitism by *M. grandii* is not higher, in spite of its synchrony with the corn borer.

I have also isolated three fungi that naturally infect the corn borer. Two of these, *Beauveria bassiana* and *Metarrhizium anisopliae* (Fig. 3), are highly pathogenic to larvae. They infect by producing spores which penetrate the body surface of the corn borer. Unfortunately their effectiveness is limited because moderate temperatures (75-85°F) and high humidity (75-100%) are required for these fungi to kill borers. This probably explains their low prevalence in populations in the field.

Although several species of bacteria inhabit the intestinal tracts of corn borers, they usually have little effect unless the borers are stressed by lack of food or adverse weather. Then, these bacteria may kill the borers.

Cultural control, which modifies the environment to the disadvantage of the borer, is an integral part of pest management. Since the corn borer overwinters in old cornstalks, infestations the following year can be reduced by plowing under or chopping up the stalks that remain at the end of the growing season or early in the spring before the adult moths emerge. I have found that early planting of corn can reduce corn borer problems (Table 2) since infestations by the first generation of borers are usually less severe than those by the second generation.

Any approach to managing corn borers depends upon reliable monitoring of populations so that pesticides or other controls can be applied only when necessary and at the proper time. Pheromone-baited traps can detect
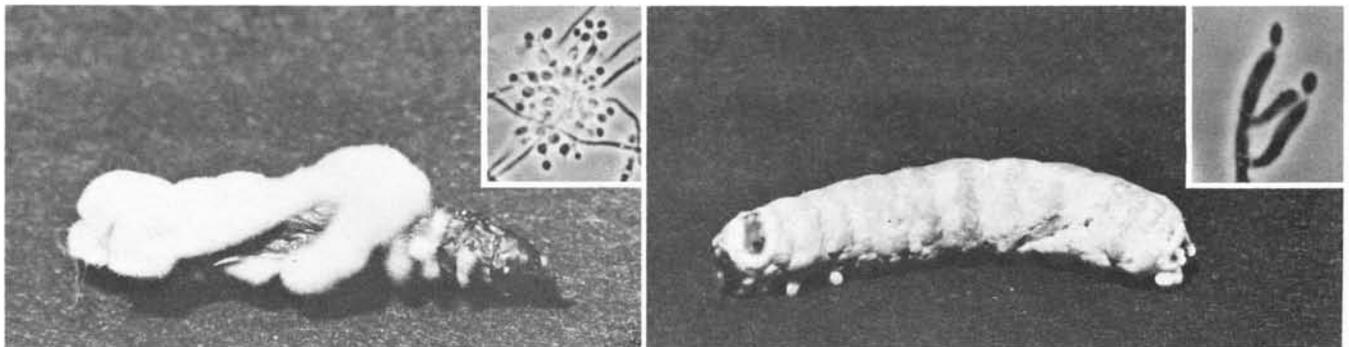

Fig. 2. *Nosema pyrausta* spores.

Fig. 3. Left, *Beauveria bassiana*—infected corn borer pupa (inset, fungal spores); right, *Metarrhizium anisopliae*—infected corn borer larva (inset, fungal spores).

the corn borer. Pheromones are lures released by female moths to attract males. Traps containing synthetic pheromones of the corn borer can reveal the presence of male moths before females have laid eggs that hatch into damaging larvae.

In 1980, tests conducted with these traps at our Lockwood Farm in Hamden showed that male moths were active during the first week in June although eggs and young borers are not evident until the end of June. Likewise, adults of the second generation are active during the first week in August, and their eggs and larval offspring appear on corn plants in a week to 10 days.

Through these and other experiments designed to increase our knowledge of the European corn borer, I

hope to refine pest management so that all available tools—chemical, cultural, and biological—may be employed to control this pest, reduce dependence on insecticides, and increase the summertime pleasure of sweet corn on the table.

Table 2. Effect of planting date on infestations of corn borer and damage to ears, 1980.

Planting	Harvest	Corn borers/ 100 stalks	% stalks infested	% ears damaged
May 8	Aug. 6	37	46	2
June 2	Sept. 2	251	92	54
July 3	Sept. 30	356	98	53

Induced resistance in plants may protect from insects and pathogens

By John L. McIntyre, J. Allan Dodds, and J. Daniel Hare

Induced resistance could provide a new means of protecting plants against fungi, bacteria, viruses, and insects.

Induced resistance may be compared to our own immune system. After we are immunized, our bodies can actively defend against certain pathogens. Although the mode of action is different, plants treated with inducing agents can also actively defend against certain pathogens to which they were previously susceptible.

Pesticides usually protect against only one group of pathogens, such as fungi or bacteria, and often against only a few members within a group. Therefore, the advantage of induced resistance is not only that it occurs throughout the plant and persists, but that induced plants also develop resistance against diverse pathogens and pests. Thus, induced resistance may provide a wide spectrum of plant disease and insect control, and supplement or replace some pesticides.

In our studies, we inoculate one or two tobacco leaves with an inducing agent, in this case tobacco mo-

saic virus (TMV). The leaves are inoculated by rubbing them with a gauze pad soaked in a TMV suspension that contains celite, an abrasive that makes minute scratches and enables the virus to enter the leaf. Within three days, the virus causes a hypersensitive response that can be seen as localized lesions on the inoculated leaf. The virus is present in only a few cells around the lesion, and causes no further harm to the plant. The TMV-inoculated leaf becomes resistant to several pathogens (challengers) within three to four days. But, more significantly, the entire plant develops resistance to the same challengers. This systemic induced resistance lasts for at least three weeks.

We used Windsor Shade 117 tobacco as the model plant in laboratory experiments. We challenged it with two fungi, a bacterium, a virus, and two insects, all of which are pests of tobacco in Connecticut. The challengers were: the black shank fungus, *Phytophthora parasitica* var. *nicotianae* and the blue mold fungus, *Peronospora tabacina*; the wildfire bacterium,

Pseudomonas tabaci; the virus, TMV; and the green peach aphid, *Myzus persicae*, and the tobacco hornworm, *Manduca sexta*.

We found that induced plants were protected, to various degrees, against all of these pathogens and pests. Protected plant leaves never developed lesions caused by the black shank pathogen; roots, the normal site of infection were also protected. The number of lesions caused by the blue mold fungus and the wildfire bacterium was reduced at least 91% on protected plants. There was also a significant but less dramatic reduction of 28% in the number of lesions caused by TMV when it was also used as the challenger. Reproduction of the green peach aphid was reduced 13%. The growth rate of the tobacco hornworm was reduced 27%. These studies were the first to demonstrate that a single agent can induce plant resistance against diverse challengers and that localized infections by a plant pathogen induce systematic resistance against insects.

We found that the plants remained protected if TMV-inoculated leaves were removed after resistance developed. Even leaves that developed after the TMV-inoculated leaves were removed became resistant.

In field plots naturally infested with *P. parasitica* var. *nicotianae*, roots of TMV-inoculated plants were resistant to this pathogen. As with our own immune system, however, booster inoculations with TMV were required to protect plants over the entire growing season. Plants inoculated one week prior to transplanting were protected early in the growing season. By the end of the season, however, disease incidence was the same in treated and untreated plants. If plants were reinoculated with TMV one month after transplanting, they were protected longer. By the end of the season, however, the disease incidence was increasing. Reinoculation both one and two months after transplanting decreased disease incidence about 50% over the entire growing season.

In other experiments we found that factors causing systemic induced resistance are phloem translocated.

Phloem tissues translocate food throughout the plant, as compared to xylem tissues, which translocate water. If we girdle the phloem but not the xylem from a TMV-inoculated leaf, the leaf develops resistance, but the rest of the plant remains susceptible. This indicates that the inducing factors do not move from the girdled leaf. If we only block the phloem going into the leaf above the TMV-inoculated leaf, systemic resistance develops except in the leaf where phloem transport is blocked. This information permits us to concentrate our research on the phloem to detect factors initiating systemic induced resistance.

Inoculation with pathogens to induce plant resistance may not be practical in the field. Thus if we can detect specific phloem-translocated factors that induce resistance it will be easier to develop artificial methods of inducing resistance. In this way, practical methods to induce resistance in the field may be developed.

Our hypothesis pictures the phloem-translocated factor not as a toxicant to challengers, but rather as a trigger that primes the plant to respond to each challenger with specific defense reactions. Evidence that supports this hypothesis is that a susceptible plant may exhibit the same resistance reaction as a plant that is genetically resistant to the same pathogen. In the susceptible plant, however, the reaction occurs later. This delay permits pathogens to become established and spread before the resistance response occurs. In the resistant plant, the more rapid response prevents spread of the pathogen. When the susceptible plant is induced to be resistant, it then responds within the same period of time as a genetically-resistant plant.

Since induced resistance has been reported in many different plant species and by several pathogens, we believe that the trigger may be a metabolite that occurs in many kinds of plants, much as our own immune system resembles that of other mammals. Thus, what we learn about induced resistance in tobacco may be applicable to other plants. In this way, induced resistance could become an important means to combat plant enemies.

Nutrients, calories, and keeping quality tested in refrigerated products

By Lester Hankin

Increased cost of food has made consumers more insistent that food be of good quality. More probably read labels to assure themselves that they are "getting their money's worth." Consumers want to be sure that perishable dairy products and other refrigerated foods will have acceptable quality for a reasonable time in the home refrigerator. They also want to know if the declarations on the label are correct and that nothing is added that does not appear on the label.

We have recently tested yogurt, juice drinks, egg

nog, and cottage cheese. Station Bulletins listing the results by brand name are available. The Connecticut State Department of Agriculture and the Experiment Station have cooperated in these studies.

Source of Products and Analysis

All samples of yogurt and cottage cheese were purchased at food stores. Juices and egg nogs were purchased at food stores or collected at bottling plants. Only juices found in refrigerated cases were tested.

Chemical and microbial tests were made at the Experiment Station using standard methods. Keeping quality was tested at the State Department of Agriculture.

Yogurt. Sales of yogurt in the United States rose from 45 grams (0.1 pounds) per person in 1954 to over 1180 grams (2.6 pounds) in 1978. People eat yogurt because it has a pleasing taste, they believe it is highly nutritious, or assume that ingestion of the live lactic acid bacteria in it gives some health benefit.

The 69 yogurt samples we tested included 32 plain (unflavored) and 37 strawberry flavored, covering 19 brands of plain and 22 of strawberry. Several brands were produced by the same processor. Although many flavors are available, strawberry was tested because it is the most popular.

We noted the age of the sample (the number of days from manufacture to purchase), the ratio of the numbers of the two kinds of lactic acid bacteria, the numbers of contaminating yeasts and molds, the acidity (a measure of tartness), and the accuracy of the claims for fat, protein, carbohydrate, and calories.

The average age of all samples was about 17 days, the range was 1 to 38 days. The code period, the number of days from manufacture to the date stamped on the carton as the last day of sale, varied from 21 to 55 days. We found that most yogurt sold in Connecticut had acceptable microbial quality. More samples of plain yogurt were contaminated with yeast and mold than strawberry. This difference is probably due to preservative in some of the strawberry yogurt. All samples contained many viable lactic acid bacteria.

The average fat content of all yogurts was about half the amount claimed on the label (Table 1). Carbohydrate, protein, and calories were near the amounts claimed. The difference in caloric content between regular and lowfat yogurts was only about 8% (Table 1). The caloric content varied little between regular yogurt (3% fat) or lowfat (about 1% fat). There is a large difference in caloric content between plain and flavored varieties. Frozen yogurt was found to be of good quality and with a caloric content about 60% higher than the fresh.

Juice Drinks. Although fruit juice drinks appear popular with persons of all ages, they are generally not aware of how much juice is in the drinks. In Connecticut the amount of juice required is set by guides established by the Department of Consumer Protection. Juice drinks (example, orange juice drink) must contain at least 30% juice; -ades, except lemon-and lime-ades, must contain at least 15% juice; lemon-and lime-ades must contain at least 12.3% juice; drinks and punch, except lemon and lime (example, orange drink) must contain at least 10% juice; lemon and lime must contain at least 6% juice; flavored drinks (example, orange flavored drink) may contain less than 10% juice; and artificially flavored products (example, artificially flavored orange drink) are not required to contain any juice.

We tested 70 juice drinks for juice content, microbial contamination, preservatives, acidity, carbohydrates, and calories. Only those declaring added vitamin C on the label were tested for this vitamin. Four iced teas were also examined.

Only six samples contained substantial numbers of yeast contaminants, and six samples contained substantial mold contamination. Labels on 33 samples declared that the preservative benzoate was used, and 14 labels declared sorbate, another preservative. Less preservative was found than was claimed in all samples. Benzoate was detected in 31 of 41 samples not declaring its use on the label, and sorbate was found in 10 samples that did not declare it on the label.

Of the 10 samples that stated vitamin C was added, five failed to meet the label claim. The caloric content ranged from 65 to 127 calories per 227 ml (8 ounces), and the average caloric content was about 100 calories per 227 ml (Table 2). Over 21% of the juice drinks did not contain the required amount of juice (Table 2).

Table 2. Average caloric content of juice drinks and the number of samples not meeting requirements for juice content.

Type of Drink	% juice required by guides	No. of samples	No. not meeting requirement	Calories per 227 ml (8 ounces)
Drinks	10	18	6	99
Lemonade	12.3	10	4	98
Flavored drinks	<10	30	0	98
Punches	10	10	5	111
Artificially flavored drinks	0	2	0	107

Cottage Cheese. In the United States the annual average consumption of cottage cheese is about 2270 grams (5 pounds) per person. Cottage cheese includes regular creamed (4% fat), lowfat (0.5 to 2% fat), dry curd (less than 0.5% fat), and flavored (fruit, vegetables, herbs and spices). Federal regulations allow 80% moisture in creamed cottage cheese and 82.5% in lowfat cottage cheese.

We collected 141 samples of various cottage cheeses representing 30 different brands. Different curd sizes (for example, large, small, tiny, California) were collected from some brands. Several brands were manufactured at the same processing plant.

We examined samples for microbial contamination, age at collection, and whether the cheese remained acceptable to the consumer to the date stamped on the

Table 1. Average nutrient composition found per 227 grams (8 ounces) of regular and strawberry yogurt.

Nutrient	Plain Regular	Plain Lowfat	Strawberry Regular	Strawberry Lowfat
Calories (no.)	145	132	239	220
Fat (gm)	3.3	1.5	2.4	1.2
Protein (gm)	8.6	9.9	7.3	7.6
Carbohydrate (gm)	20.5	19.7	47.0	45.0

carton as the last day of sale. We also tested for preservatives; sodium content; percentages of fat, protein, carbohydrate, and moisture; and calories per 133 grams (4 ounces).

Code periods (days from manufacture to last date of sale stamped on the carton) ranged from 13 to 60 days. The average age at collection was 16 days with a range from 5 to 39 days. About a fifth of the samples were not acceptable by the end of their code period. Five samples past their code dates (outdated) were of acceptable quality. Four samples contained visible microbial growth on the surface when purchased.

The average fat content of all samples was 107% of the amount claimed. The protein and caloric contents averaged over 90% of the amount claimed. Lowfat cottage cheese averaged 50% more fat than claimed on the label (Table 3).

Table 3. Average percent of label claim found for nutrients in 141 cottage cheese samples.

Nutrient	Type of cottage cheese		
	Regular	Lowfat	Flavored
Calories	91	96	91
Fat	101	150	85
Protein	96	100	92
Carbohydrate	77	89	104

The preservative sorbate was found in 23% of the samples although only 11% declared its use on the label. In two cases, sorbate was declared but not found.

Since some individuals restrict salt intake, we determined the sodium in all samples. The average sodium content of samples claiming that salt was added was 472 mg per 113 grams (4 ounces).

Egg Nog. Egg nog is a seasonal product sold from about Thanksgiving to New Year's Day. We tested 28 brands of egg nog for keeping quality, microbes and nutrients.

The flavor quality of all samples remained acceptable to the code date (last day of sale stamped on the carton) assigned by the processor. Code periods (days from manufacture to code date) ranged from 10 to 38 days. Fat content ranged from 5.3 to 9.5% and calories per 227 grams (8 ounces) ranged from 265 to 461, averaging 321. About 68% of the samples met the requirements for at least 1% egg yolk solids. No preservatives were found. Microbial quality was generally satisfactory.

Specific Results

Although I have discussed only general trends, many will want to know about the brands that they purchase. Information about specific brands is available. Copies of the following bulletins may be requested from Publications, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, CT 06504. The titles are:

Bulletin 785   Quality of Yogurt
Bulletin 790   Quality of Juice Drinks
Bulletin 791   Quality of Cottage Cheese
               and Ricotta Cheese
Bulletin 793   Quality of Egg Nog

Detecting aflatoxins as contaminants in milk and other food products

By Richard P. Kozloski

Twenty years ago the existence of the powerful cancer-producing compounds known as aflatoxins wasn't even suspected. Since their discovery in 1960, during the investigation of deaths of animals fed moldy peanut meal, these compounds have been found in foods intended for human consumption.

Various nuts and grains are particularly susceptible to aflatoxin producing molds such as *Aspergillus flavus*. Of the nuts, peanuts have been shown to present a special problem; cracking of the shell during harvesting promotes the growth of aflatoxin producing molds. One badly contaminated nut in a lot of 50,000 peanuts, can raise the average aflatoxin level beyond the permitted level. Because of this problem, peanuts are carefully inspected for aflatoxin content.

Animal feeds, such as corn silage, can also become

Aflatoxins

The major aflatoxins found in grains and nuts are aflatoxins B_1, B_2, G_1, and G_2. The terms are derived from the color of fluorescence under long-wave ultraviolet light, the B is for a bluish and the G is for a greenish fluorescence. The numerical subscripts refer to the order of their separation by thin layer chromatography.

Some aflatoxin-caused deaths have occurred in Africa and Asia. Reyes syndrome, a sometimes fatal disease of children, may also be related to aflatoxins.

Aflatoxins damage the body when the liver attempts to detoxify them by modifying them chemically. One product binds to the DNA and other important compounds of the liver cells. The cells no longer function; consequently the ability of the liver to remove toxic substances is severely damaged. At lower concentrations liver function is not affected, but cancer can result from the damage to the DNA.

The liver acting on aflatoxin B_1 can produce another aflatoxin, designated M_1. The M stands for milk, since this aflatoxin shows up in the milk of cows that have ingested aflatoxin B_1.

contaminated with aflatoxin. When cows eat contaminated silage, the milk produced by these cows can contain aflatoxin, and therefore pose a hazard for people.

The U.S. Food and Drug Administration will take legal action to remove milk from the market if aflatoxin M_1 exceeds a level of 0.5 part per billion (about ½ ounce per 10 million gallons). One consideration in setting such a low limit is that children consume large amounts of milk. The low limit challenges analytical chemists to devise sensitive, inexpensive, and rapid tests for aflatoxin M_1 in milk.

Aflatoxins are now detected by thin layer chromatography (TLC). In TLC a thin coating of adsorbent material such as silica gel is formed on a glass plate. A small spot of extract is deposited near the bottom of the plate. The plate is then placed in a tank with its bottom edge in contact with solvent. As the solvent mixture is drawn up the adsorbent coating by capillarity, the different components of the spot are carried along to different degrees. The plate is then dried and examined under longwave ultraviolet light. Aflatoxin components are characterized by the distance they travel with a given solvent and by the color of their fluorescence.

Frequently this first separation fails to separate aflatoxins from impurities. By having a second solvent migrate across the plate in a direction perpendicular to the direction of the first solvent, I can usually obtain a good separation. I have used this technique to analyze for aflatoxins in chicken feed and urine.

The separation of aflatoxin M_1 from three ounces of milk requires one quart of organic solvents. Methyl alcohol breaks down the milk emulsion and dissolves the aflatoxin. After filtering, the fat is extracted with hexane. An extraction into chloroform allows the aflatoxins to be concentrated by evaporation.

With conventional plates the TLC separation took over one hour. Much skill and patience were required to apply samples to the plates. By using high performance TLC plates with a special zone for applying the sample, I have overcome both problems. High performance TLC plates differ from conventional plates in that the particle size range of the adsorbent coating is

narrow. This improves resolution. Now, a good separation can be achieved by letting the solvent travel only two inches. The smaller spot size also increases detection sensitivity.

The special zone consists of a layer of diatomaceous earth at the base of the plate. The dissolved sample is deposited on the zone as a line in the direction that the solvent will travel. After the sample dries, the TLC plate edge is placed in contact with a solvent. The solvent dissolves the sample and moves it to the silica gel region of the plate, where the sample is resolved into its components.

Besides exploring new solvent systems to improve the separation of the aflatoxins, I have developed new TLC techniques. I have reduced the amount of fat in the extract spot by first using a solvent that does not move aflatoxin. This prevents the aflatoxin spot from smearing. Another novel technique, which reduces spot size, is to flow solvent toward a spot from opposite sides. This causes the spot to become concentrated where the two solvent fronts meet.

I have greatly simplified extraction of aflatoxin from milk by using a device called a SEP-PAK. The SEP-PAK is a small cartridge that contains silica gel with a thin organic layer bonded to its surface.

When milk passes through the cartridge, the aflatoxin and other dissolved organic compounds are adsorbed. The milk fat, which causes a problem in the old method of TLC, flows through and can be discarded. The protective layer of protein, which keeps fat from coagulating, prevents the fat from being adsorbed. Some weakly adsorbed milk constituents are washed from the SEP-PAK with a 10% acetonitrile solution. The aflatoxin is then extracted from the SEP-PAK with a 30% acetonitrile solution. After I extract the aflatoxin from the solvent and concentrate it by evaporation I analyze it by TLC. Less than one ounce of organic solvents is used and the time required for an analysis is reduced from half a day to about one hour. Now that I can detect the presence of aflatoxin M_1 at a level of 0.05 parts per billion, I am now developing methods to measure the exact amount of aflatoxin in milk.