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*David E. Stilwell
checks tapped
maple tree*



Controlling black vine weevils

Tracing lead in maple syrup

Testing for Cyclospora

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Several methods reduce insecticide use in control of black vine weevils

By Richard S. Cowles

Black vine weevils are one of the most destructive insect pests affecting production of nursery crops, small fruit, and hops. Adult beetles feed extensively on foliage, causing unsightly notches. Root damage from larval feeding is usually more important to the health of the plant, hence the black vine weevil and its close relatives are called "root weevils." The legless larvae first feed on small roots. Large larvae may girdle the main roots, thereby killing the plant. During 6 years of evaluating options for managing this pest, I found different techniques are appropriate for different situations.

Black vine weevil adults are 8.5-11.5 mm long (1/3-1/2 inch), jet black, with a beaded appearance and patches of yellow hairs on the back (Fig. 2). Adults will drop to the ground and play dead when disturbed. Adult females (there are no males) feed on leaves at night for about a month before laying eggs in the soil. Adult emergence and peak feeding activity coincide with late bloom and fruit ripening of strawberries. Females lay 200-600 eggs per year, allowing populations to increase rapidly.

Although flightless as adults, black vine weevils have successfully exploited the nursery trade to become world travelers. Larvae feeding on roots are hidden, so they may



Figure 2. The black vine weevil adult has a short snout, elbowed antennae, and patches of light-colored hairs on its back.

be unwittingly sold with plant material, leading to new infestations. Black vine weevils feed on most non-grasses, so food is abundant.

Their ability to feed on plants toxic to humans, such as yews and rhododendrons, has preadapted them to also detoxify man-made insecticides. Many insecticides therefore fail to kill adults. I have investigated three management possibilities for control of this weevil: exclusion, biological control, and bait-formulated insecticides.

Exclusion barriers take advantage of the fact that black vine weevil adults cannot fly. In 1994 I field tested several lubricants which had prevented black vine weevils from climbing aluminum barriers in the laboratory. My test sites were heavily irrigated container-grown areas within three cooperating nurseries. I buried the bottom 2 inches of aluminum flashing (6 inches wide), applied lubricant to the top 2 inches, and then left them to weather for 1-4 weeks. Lithium grease, which was 100% effective in preventing black vine weevils from climbing aluminum, was the only treatment not eroded from the aluminum by irrigation, and therefore the only one that can be used for exclusion. (Table 1)

Such exclusion barriers, which control root weevils without the use of chemical pesticides, can last several years. However, they are relatively expensive to install: \$328 for the material to enclose 1 acre. Other impediments



Figure 1. Richard S. Cowles testing grease-coated barriers.

are the need to maintain a grease barrier and incompatibility with operations that require large equipment in fields.

Biological control of black vine weevil can be accomplished with commercially available insect pathogenic nematodes, including the species *Steinernema carpocapsae*, *S. feltiae*, and *Heterorhabditis bacteriophora*. Unlike plant pathogenic nematodes, which feed on and damage plants, insect pathogenic nematodes feed solely on insects. The infective juvenile nematodes are applied as a soil drench. They enter weevil larvae through body openings and release symbiotic bacteria, which in warm soil kill in 2-3 days. After two to three generations (depending on the size of the larva infected), young nematodes exit from the dead larva and seek new hosts.

Although nematodes can provide excellent control of black vine weevil larvae or pupae under greenhouse conditions, they often give poor results when applied in the field. Likely explanations for this difference are that the soil is too cold, too dry, or too compacted for the nematodes. Since container-grown ornamentals are typically grown in an artificial medium and in black pots that capture the sun's heat, such plants could be well suited for biological control with nematodes.

I infested 16 container-grown azaleas with 10 fully-grown black vine weevil larvae each. Half of the pots were treated with *S. carpocapsae*, and half with *H. bacteriophora* (10,000 nematodes per pot). Fourteen days later the percent mortality was 68 ± 5.6 for *S. carpocapsae* and 83 ± 4.9 for *H. bacteriophora*. The number of nematodes exiting black vine weevil larvae was greater with *H. bacteriophora* (25,000 vs. 12,000). *H. bacteriophora* appears to be more effective.

My 1996 field results in strawberries suggest that these nematodes may require up to 4 months of warmth to reach their full biological potential (Fig. 3). The data have been

averaged across species of nematode applied; there was a similar response with each nematode tested. Nematodes were applied at a rate of 0.5 billion per acre (*H. bacteriophora*), 1 billion per acre (*S. feltiae*) or 3 billion per acre (*S. carpocapsae*). The damage threshold for strawberries is approximately four larvae per 5 liters of soil. Appropriate application timing is when weevil eggs have hatched (mid-August) or as soon as the soil warms in the spring (early- to mid-May). Soil must be moist for nematodes to be effective.

Foliar sprays of insecticides are the standard practice to manage black vine weevils. However, these also kill beneficial parasites of scales and predators of mites. Insecticides formulated as baits were first tested against black vine weevil in the late 1920's, then neglected for about 60 years. My research has focused on insecticides formulated into dried apple pomace, the waste remaining after apples are pressed to make cider. In laboratory tests, I found that a bait must be

Table 1. Effectiveness of lubricants for preventing black vine weevil adults from climbing a sheet of aluminum. Lubricant was weathered for 1-4 weeks at cooperating nurseries; data have been averaged over all weathering groups and represent means for 12 observations.

| Lubricant | Beetles climbing barrier (%) (mean \pm SE)* |
|----------------|--|
| Oil (10W-40) | 44 \pm 9 a |
| Oil (WD-40) | 53 \pm 10 a |
| Lithium grease | 0 \pm 0 b |
| Non-lubricated | 44 \pm 9 a |

Means followed by the same letter are not significantly different, $P < 0.05$, Student-Newman-Keuls test.

Table 2. Materials tested in the laboratory for incorporation with apple pomace to control adult black vine weevil. Mortality was measured for three replicates of 15 beetles allowed to feed for 7 days on yew foliage or 2 g of bait (rewetted every 3 days).

| Material | Chemical | Active ingredient (%) | Mortality* (%; mean \pm SE) |
|--------------|--------------|-----------------------|-------------------------------|
| Orthene | acephate | 3.3 | 66 \pm 10 a |
| Turcam | bendiocarb | 3.25 | 73 \pm 6 a |
| Talstar | bifenthrin | 0.25 | 57 \pm 7 a |
| Pylon | chlorfenapyr | 0.75 | 73 \pm 7 a |
| Cryocide | cryolite | 20 | 53 \pm 18 a |
| EXP60720A | fipronil | 0.14 | 73 \pm 11 a |
| blank pomace | - | - | 6 \pm 4 b |

*Means followed by the same letter are not significantly different, $P < 0.05$, Student-Newman-Keuls test.

moist to be eaten by black vine weevils. Several insecticides are good prospects for incorporation into baits (Table 2). A 1996 field trial in strawberries demonstrated this potential. I compared standard foliar sprays with bait formulated cryolite (a low-toxicity mineral) and a new low toxicity pesticide, fipronil. While foliar sprays with labeled products caused no significant mortality, the baits and the fipronil spray caused significant reductions in weevil numbers.

My research shows that appropriate methods for black vine weevil control vary according to the situation. In container-grown nurseries, especially in propagation areas, so few larvae can be tolerated that preventative chemical treatments of potting mix or mid-August application of nematodes are warranted. In field-grown ball and burlap nurseries, pesticide labels do not permit sufficient material to be mixed in the soil for adequate control of larvae, so biological control with nematodes and bait formulated insecticides are promising alternatives. Berry growers could use nematodes because chemical control of adults may not be effective with currently registered insecticides. For landscape plantings, exclusion and the use of nematodes are options to compare with commonly used insecticides.

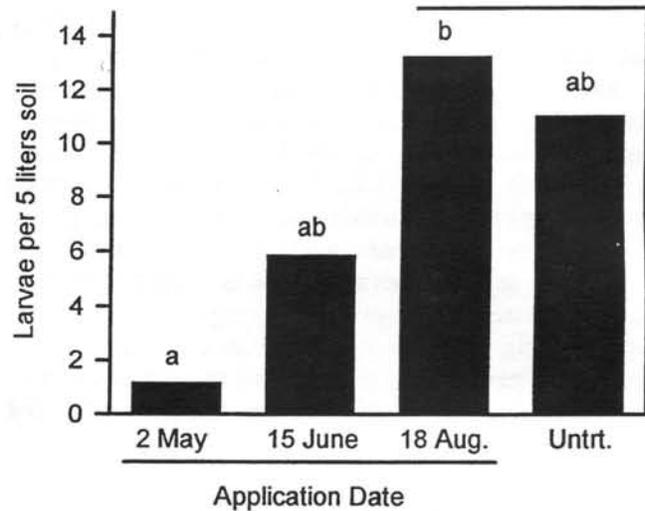


Figure 3. Number of black vine weevil larvae in soil samples taken September 27-October 10, 1996, from strawberry plots treated earlier with insect pathogenic nematodes. Columns with the same letter are not significantly different ($P < 0.05$, Student-Newman-Keuls test).

Processing equipment influences lead in maple syrup

By David E. Stilwell and Craig L. Musante

In 1989, higher than average lead contents compared to other dietary components were found in maple syrup produced in Canada. Subsequent testing has indicated that the problem was industry wide. We have tested maple syrup for four seasons (1994-97) and have been investigating and identifying possible sources of lead in the syrup.

During the four seasons, we tested 117 grade A maple syrup samples from about 45 of 120 Connecticut producers. Some were obtained over several seasons, while some were obtained only for one season. Replicate samples were received from two producers in 1994, three producers in 1995, 25 producers in 1996, and 12 in 1997. The samples came from retail stock in producer-supplied containers (typically plastic), ranging in size from 84 to 475 mL.

In addition, to compare the lead concentrations in maple syrup to other syrups, eight commercially available table (corn) syrups and ten molasses products were purchased. The syrup samples were prepared for analysis by acid digestion and the lead (Pb) content determined using a graphite furnace atomic absorption spectrophotometer.

Table 1 summarizes our findings for the lead content in maple syrup. If replicate samples were taken the results were averaged. Over the 4 years, 36% of the producer-samples were less than 100 $\mu\text{g}/\text{kg}$ in lead while 8% exceeded the 500 $\mu\text{g}/\text{kg}$ lead advisory limit recently established by the State of Vermont. The lead content in the syrups was highest in the 1995 samples. Five in 1995 (14%) exceeded 500 $\mu\text{g}/\text{kg}$. In 1996 one producer-sample exceeded the 500 $\mu\text{g}/\text{kg}$ level for lead, and in 1997 none exceeded this level. Since 1995 the median lead in the samples dropped from 338 to 91 $\mu\text{g}/\text{kg}$. The overall average for the 4-year study reported here (245 $\mu\text{g}/\text{kg}$ lead), is lower than the level obtained from 27 producers in Canada in the 1989 survey.

The results for the lead levels in commercially available table (corn) syrups and molasses products are given in Table 2. The lead content in only one of the eight corn syrup samples was above the detection limit, while lead was detected in six of the ten molasses samples. The lead content of the blackstrap molasses was similar to that found in the maple syrups, but did not exceed the Vermont advisory level.

How maple syrup is processed

Maple syrup is a truly natural product which is made by concentrating the watery sap into a rich syrup by boiling. The short, 4-8 week season starts in late winter to early spring. During this time maple sap flows in harvestable amounts only when below freezing night-time temperatures are followed by above freezing daytime temperatures.

The first step in the syrup producing process is to collect sap. At the grove site, sap from trees flows through taps into containers. Some producers use plastic or metal buckets, while others use a network of plastic tubing to feed sap into large containers. In general, the next step is to transfer the sap from the grove containers into large plastic transport tanks situated on a truck or tractor. Many producers use many varieties of pumps to transfer the sap. Some producers then clarify and filter the sap by passing it through a diatomaceous earth filter. Finally, the sap is pumped into a stainless steel holding tank. In addition, some producers pre-process the sap before it enters the boiling equipment. These processes include devices for pre-heating the sap (copper tubing pre-heaters, and Steam-Aways) or concentrating the sugar in the sap by reverse osmosis.

The sap is then processed into maple syrup by vigorous boiling in a series of metal pans. The concentration factor for sap to syrup is around 40:1. When this concentration factor is achieved, the syrup is filtered, finished to the required viscosity, and graded by color before bottling.

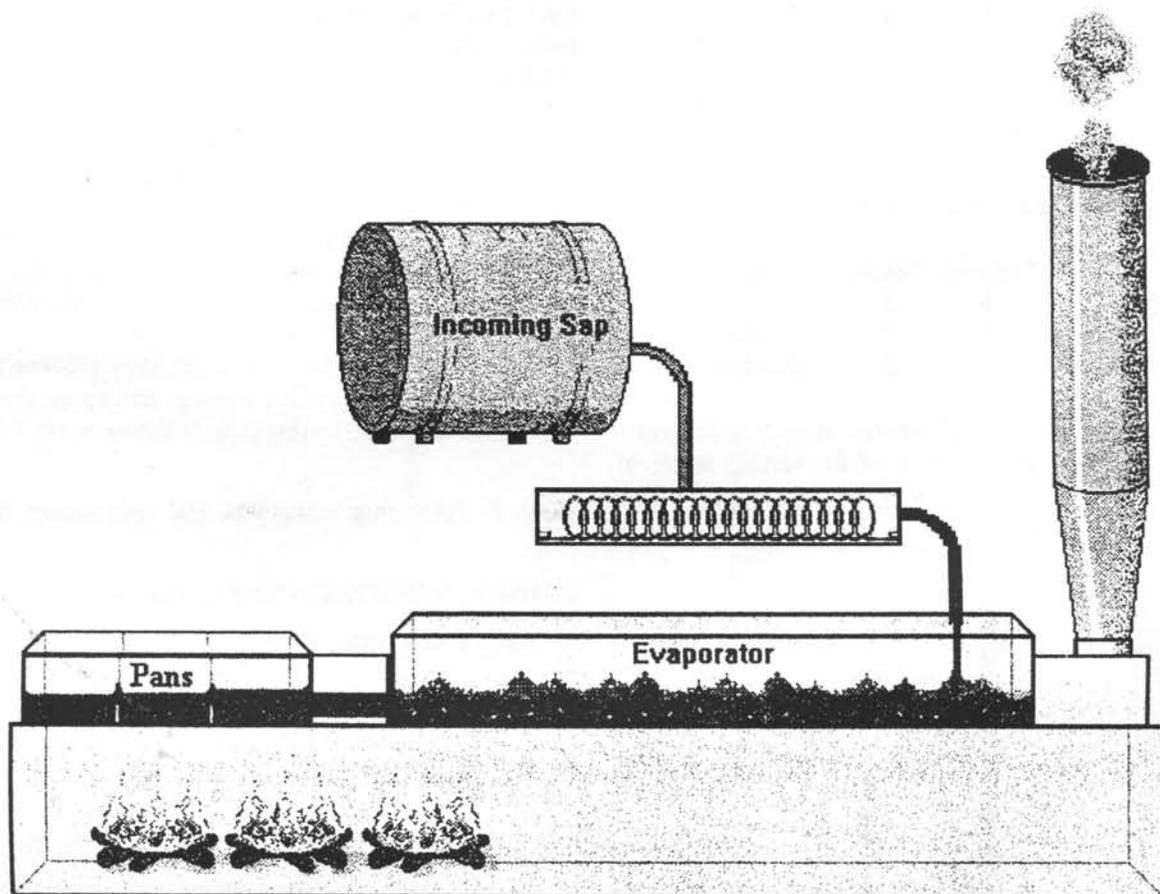


Figure 1. Schematic of maple syrup processing equipment. Sap is fed into the evaporator from a stainless steel holding tank. In many cases this incoming sap is preheated by passing it through coiled copper tubing which is heated by the steam generated by the boiling sap in the evaporator. The sap is fed by gravity into the pans. The thickening syrup winds through the pan partitions and is drawn out after it reaches the desired density. Most syrup processors are wood-fired.

Table 1. Lead content ($\mu\text{g}/\text{kg}$) in maple syrup.

| Year | Samples/ Producers | Lead Contents ($\mu\text{g}/\text{kg}$) | | |
|-------|-----------------------|---|------|--------|
| | | Range | Avg. | Median |
| 1994 | 23 (17) | 46-469 | 199 | 159 |
| 1995 | 21 (18) | 38-948 | 378 | 338 |
| 1996 | 50 (25) | 22-1966 | 222 | 129 |
| 1997 | 23 (12) | <20-354 | 161 | 91 |
| Total | 117 (72) | <20-1966 | 245 | 151 |

Producers with samples ($\mu\text{g}/\text{kg}$) in a given range.

| Year | Range ($\mu\text{g}/\text{kg}$ Lead) | | | | |
|-------|---------------------------------------|---------|---------|---------|------|
| | <100 | 101-200 | 201-350 | 351-500 | >500 |
| 1994 | 4 | 7 | 3 | 3 | 0 |
| 1995 | 4 | 2 | 3 | 4 | 5 |
| 1996 | 11 | 6 | 4 | 3 | 1 |
| 1997 | 7 | 0 | 4 | 1 | 0 |
| Total | 26 | 15 | 14 | 11 | 6 |

Table 2. Lead content ($\mu\text{g}/\text{kg}$) in other syrup products.

| Product | Samples | Brands | Range | Average |
|---------------------|---------|--------|---------|---------|
| Corn Syrup | 8 | 8 | <20-32 | <20 |
| Molasses | 7 | 7 | <20-255 | 68 |
| Blackstrap Molasses | 3 | 2 | 106-490 | 238 |

To determine the potential sources of lead in the syrup, maple sap samples representative of the various stages of

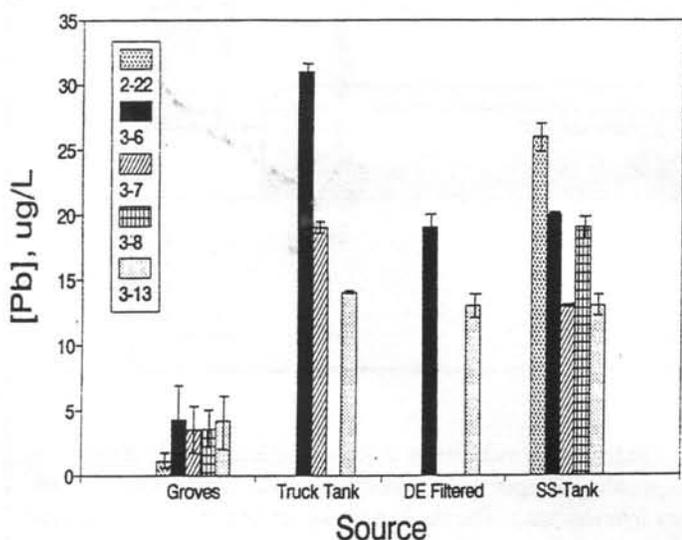


Figure 2. Lead concentration in sap from a producer who used a bronze gear pump to transfer sap.

collection, storage, and processing were analyzed for lead, and some for copper and zinc.

The first problem was to determine how much lead is in sap taken directly from trees. During the 1995 season we collected 35 sap samples in plastic containers at maple groves. For the 1996 and 1997 season, sap tapped directly from 10 trees was sampled (once in 1996 and up to four times in 1997). Lead ($\mu\text{g}/\text{L}$) in the sap samples from plastic grove tanks ranged from <0.5 to 5.4 and averaged 1.1. Lead ($\mu\text{g}/\text{L}$) tapped directly from trees ($n=41$) ranged from <0.5 to 7.8 $\mu\text{g}/\text{L}$ and averaged 2.6 ± 1.8 . These results suggest that the lead content in sap directly from the trees is low but measurable.

From 1995 to 1997 we collected and analyzed sap samples from seven producers. The results of the analysis of these samples were used to identify contaminant sources. An example is in Figure 2. The lead values for the grove samples represent an average of all samples ($n=6-10$) taken at the various grove sites at a given date. The lead level of all grove site samples taken from this producer averaged 3.3 $\mu\text{g}/\text{L}$. The results show a marked and reproducible increase in lead in the sap after a bronze gear pump was used to transfer sap into the truck tank. To verify that the pump was the contaminant source, approximately 20 liters of sap were placed into a plastic container, recirculated by the pump, and periodically sampled (100 mL) for 6 minutes. Both the copper and lead contents increased more than 300%. These results demonstrate that the bronze gear pump can contaminate sap with lead and copper.

In addition, sap samples from six other producers taken after various sap collection, transfer and processing steps were tested for lead. The results are summarized in Table 3.

Table 3. Processing equipment and relationship to lead in sap.

EQUIPMENT SHOWN TO CONTAMINATE SAP WITH LEAD

- Bronze gear pumps.
- Lead soldered metal buckets, and other galvanized containers soldered with lead.
- Lead solder and bronze fittings in the pre-heater assembly.

EQUIPMENT SHOWN NOT TO CONTAMINATE SAP WITH LEAD

- Metal buckets constructed using lead free solder
- Most centrifugal pumps not constructed with bronze
- Steam-Away (a device used to heat and concentrate sap)
- Pre-heaters constructed with lead free solder

Because maple sap is acidic (pH 3.4-6.6) and in the presence of oxygen, it has the potential to react with many met-

als; therefore contact with lead-containing metals should be avoided when sap is collected and processed.

Many evaporators and pans were fabricated using lead solder; thus, the evaporation and boiling processes have the potential to add lead to the syrup. Moreover the lead in the sap could be concentrated into the syrup, as the concentration factor for sap to syrup is around 40:1.

We found that our results were affected by a precipitate that invariably forms during the evaporation. This solid matter, commonly referred to as "sugar sand", is transferred in the solution phase until it is filtered out at the final step. To distinguish between analytes in the solution and solid phase, the following protocol was developed. First, the process batch sample was vigorously shaken, and two replicates were weighed out for digestion. These samples, containing both the solid and solution phase, were denoted as "mixed-phase." Separation of the phases was accomplished by centrifuging. These samples were denoted as "solution-phase."

Analyses indicate that much of the lead precipitates from solution during processing, as shown by the higher lead levels in the mixed phase samples until the final filtering. For example, the average lead concentration in one evaporator sample averaged 4270 $\mu\text{g}/\text{kg}$ in the mixed phase samples,

and only 221 in the solution phase samples. Also, a sample of the filter residue collected prior to bottling the final syrup contained 33,000 $\mu\text{g}/\text{kg}$ lead while the final syrup contained 575 $\mu\text{g}/\text{kg}$. The amounts of lead in the process stage differ in the mixed phase results between the two sample sets, while in the solution phase they were similar. During processing, the build-up of lead-laden sugar sand is likely to account for the increase in mixed phase lead from the second sample set.

The solution phase data show that the lead content was minimum at the evaporator stage, and then slowly increased. The reasons for the gradual increase are not known, but decreasing pH may be one factor. Another factor could be leaching of lead from the solder used in fabricating the equipment (for this producer, the lead content of the solder used in the evaporator and pans ranged from 50-60% lead).

Commencing with the evaporator stage, we found that the concentration of phosphorus was much lower in the solution phase than in the mixed phase. Therefore we concluded that lead may have precipitated out in the form of lead phosphate, which is highly insoluble. This partitioning of lead into solution and solid phases must be taken into account in order to properly identify sources of lead contamination in the final product.

Cyclospora does not contaminate Connecticut-grown strawberries

Charles R. Vossbrinck and Theodore G. Andreadis

Cyclospora became news last spring and summer and again this year when a number of human infections were

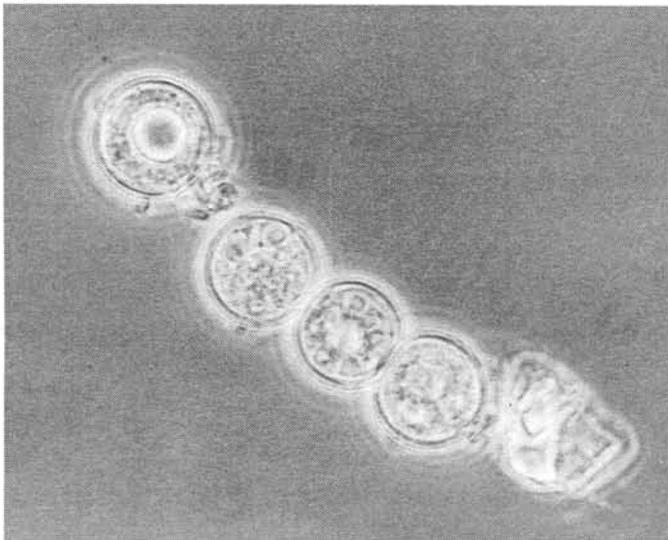


Figure 1. *Cyclospora cayetanensis*, about 1400X.

confirmed in the United States. Initially, contaminated strawberries were thought to be the source. The disease is caused by a protozoan parasite, *Cyclospora cayetanensis*. This single celled organism (Fig. 1) is about 1/100 millimeter (8-10 microns) in diameter and reproduces in the small and large intestines of humans. Symptoms usually appear 7 days after consumption of contaminated food and include nausea, extreme fatigue, intestinal cramps, loss of weight, and watery diarrhea. The delay between consumption and onset of symptoms makes tracing the source difficult because people often forget what they ate the previous week. Before last year, few cases of Cyclospora were reported. In 1996 however, 978 laboratory-confirmed cases of Cyclospora were reported in the United States and Canada. This included 38 cases in Connecticut in 1996 and 17 in 1997. All of these cases were acquired between the second week in May and the first week in June.

In response to this outbreak and its suspected relation to strawberries, we examined strawberries collected from Connecticut growers to see if we could find Cyclospora.

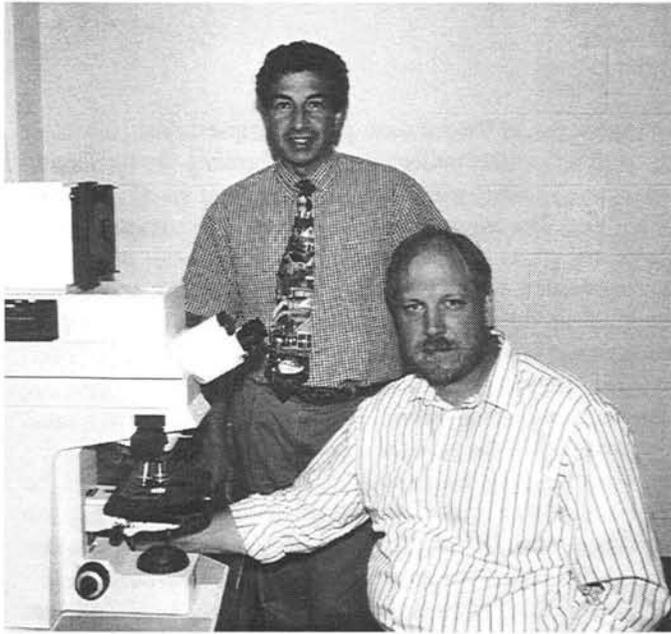


Figure 2. Theodore G. Andreadis (L) and Charles Vossbrinck (R).

Twenty-four samples were collected by Experiment Station plant inspectors from seven different sites (Avon, Windsor, So. Windsor, So. Glastonbury, Somers, Shelton and Guilford). The strawberries were frozen and later tested.

Ten strawberries from each sample were soaked for ½ hour in 500 milliliters of water. The samples were shaken briefly and the water was poured off and filtered to remove large particles. The water was then centrifuged at 500 g for 15 minutes to concentrate any material washed from the strawberries. The pellet was re-suspended in 1 ml of water containing 2.5% potassium dichromate to enhance spore identification. We then examined two portions of each sample for *Cyclospora* by phase contrast and fluorescence microscopy at low and high magnification. Fecal samples containing *Cyclospora* were obtained from Dr. Charles Sterling of the University of Arizona to compare with material washed from the strawberries.

We did not find *Cyclospora* on the strawberries from Connecticut. Further, the latest report from the Centers for Disease Control indicates that the source of last summer's outbreak was Guatemalan raspberries which had been irrigated or sprayed with water contaminated by human waste.

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