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*Carol Lemmon checks bees*

New tick-associated disease in state

Specialty crops being tested by Station

Anthracnose fruit rot of strawberry

Mites infesting honey bees

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION,

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# Babesiosis is a new tick-associated disease of humans in Connecticut

**By John F. Anderson, Eric D. Mintz, Joseph J. Gadbaw, and Louis A. Magnarelli**

The first human case of babesiosis found in Connecticut occurred in 1988. Twelve cases acquired by tick bite and one case contracted by blood transfusion were documented from 1988-1990, which is the largest number of human cases of babesiosis reported from mainland United States.

Persons living in Stonington, Old Lyme, and Montville contracted babesiosis from tick bites in July through October. Patients ranged in age from 61 to 95 years; two died with active infections, and a third died from chronic obstructive pulmonary disease shortly after antibiotics had suppressed the *Babesia* parasites. Eight other patients were hospitalized and recovered without incident. A 46 year-old man without a spleen accidentally became ill with babesiosis following a transfusion of blood. The blood was donated by a person living in Old Lyme who exhibited no signs of illness. *Babesia microti* was subsequently identified in the blood from the donor. The recipient recovered following treatment.

We confirmed the presence of *B. microti* by isolation and determined that the parasite was prevalent in white-footed mice living in the yards of the patients. To attempt isolation of the parasite from humans, we inoculated 0.5-1.0 ml of uncoagulated blood, drawn from suspected patients, into hamsters. At 2 week intervals for 6 weeks after inoculation, a drop of blood was obtained from each inoculated hamster, smeared on a glass slide, and the cells stained. Cells were examined for parasites at a magnification of 1,008X with a compound microscope. The *Babesia* parasite was isolated from seven of 12 patients tested.

To determine the prevalence of infection of *B. microti* and the Lyme disease-causing spirochete, *Borrelia burgdorferi*, in white-footed mice inhabiting the yards of patients and in forested areas in other Connecticut towns, mice were captured in Sherman box traps and tested in the laboratory. The presence of *Babesia* parasites was determined as described above for humans, except 0.1 to 0.5 ml of mouse blood was inoculated into each hamster. The test for presence of Lyme disease-causing spirochetes required that we inoculate various mouse tissues, such as skin, spleen, kidney, and bladder, into a special liquid, cell-free culture medium called Barbour-Stoenner-Kelly. Two to 6 weeks after inoculation, cultures were examined for spirochetes by dark-field microscopy.

*Babesia* parasites and Lyme disease spirochetes were recovered from mice captured in the yards of the eight patients. Forty-six percent and 64% of the 59 mice tested were infected with *B. microti* and *B. burgdorferi*, respectively. Forty-two percent of the mice were infected with both organisms. Based on these findings, we conclude that most of the patients were probably exposed to ticks in their own yards.

We suspected that some of the patients with babesiosis may also have been exposed to the spirochetes that cause Lyme disease. Sera from seven patients diagnosed with babesiosis were tested for antibody to the Lyme disease-causing spiro-

## Babesiosis in humans

The first human case of Lyme disease caused by the spirochete *Borrelia burgdorferi* and the first human case of babesiosis caused by the protozoan parasite, *Babesia microti*, in the United States were described from different geographical areas in 1970. Lyme disease, then known as erythema chronicum migrans, was diagnosed for a grouse hunter in Wisconsin. Human babesiosis was identified in a summer resident on Nantucket Island in Massachusetts.

The majority of the more than 200 humans diagnosed with babesiosis from 1970 through the late 1980s lived on or visited islands of Massachusetts, Rhode Island, or New York. Exceptions included two cases from Cape Cod in Massachusetts and two cases from Wisconsin.

Both pathogens were later found to occur naturally in white-footed mice and to be conveyed to humans by the bite of the same tick, *Ixodes dammini*, the so-called deer tick.

Humans acquire *Babesia microti* infections primarily from bites of nymphal ticks that previously had fed as larvae on white-footed mice. During its 2-year life cycle in Connecticut, *I. dammini* develops through four stages of growth: egg, larva, nymph, and adult. Fully-fed adult female ticks lay their 2,000 eggs in a single cluster on the ground during spring. Larval ticks hatch from eggs in mid to late summer and feed on mammals of all sizes and on birds. When full, the ticks drop to the ground, where they molt into nymphs the following spring. The nymphs feed until full on mammals and birds in late spring and early summer, drop to the ground, and molt into adult ticks in fall. Adult females feed on large and medium sized mammals in the fall, on warm days during winter, and during early spring of the next year. All feeding stages of *I. dammini* parasitize humans.

Clinical signs of human babesiosis range from no symptoms to severe, including death. The parasite invades and destroys red blood cells. Symptoms include intermittent fever, chills, drenching sweats, joint pain, muscle ache, malaise, headache, loss of appetite, anemia, and weight loss. A skin rash is not associated with babesiosis as it is with Lyme disease.

Babesiosis is more severely expressed in persons over 60 and persons without spleens. Diagnosis is based on clinical symptoms, the finding of *Babesia* parasites in the patient's red blood cells, a significant serum antibody (titer >1:256) to *B. microti* determined by an indirect fluorescent antibody staining method, and isolation of *B. microti* from human blood in hamsters. The antibiotics clindamycin and quinine are often used for treatment. Exchange transfusion has been used concurrently with chemotherapy in profoundly ill patients.


**Figure 1. Co-authors John F. Anderson (left), Louis A. Magnarelli (center), and Joseph J. Gadbaw (right) at Lawrence and Memorial Hospital in New London.**

chete in an ELISA test. Five had significant reactions (titers $\geq$1:640). These patients may have been infected with both organisms following a single tick bite, or alternatively they may have been bitten by more than one tick.

Mice captured in Lyme, East Haddam, and West Hartford, where no human cases of babesiosis have been reported, were also found infected with *B. microti*. We believe that the *Babesia* parasite will likely spread to other areas of Connecticut where *I. dammini* ticks are common, and, that in time, humans may acquire this infection in areas outside of southeastern Connecticut.

We conclude that residents increasingly will need to be educated on the symptoms of both Lyme disease and human babesiosis and the biology and control of the tick, *Ixodes dammini*.

*Bonnie Hamid, Jim Ayers, and Neil Infante assisted*

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### Plant Science Day to be held August 12

The annual Plant Science Day open house will be held at the Experiment Station's Lockwood Farm in the Mt. Carmel section of Hamden from 10 a.m. to 4 p.m. on Wednesday August 12.

John J.C. Herndon, deputy chief of staff to Governor Lowell P. Weicker Jr., will be the main speaker at 11:30.

There will be short talks by Station staff, exhibits, and experimental plots on display.

# Experiment Station tests specialty crops for Connecticut farmers

**By David E. Hill**

In 1983, the Experiment Station began to investigate new crops to provide opportunities for farmers during a time of changing agriculture. New crops include broccoli and cauliflower that have been grown in Connecticut but have experienced a resurgence in interest, and niche crops, which are popular specialty crops whose culture must be adapted to Connecticut's soil and climate. The selection of crops was largely based on their potential for high economic value and increased demand due to an expanding market.

*Artichokes*

The first crop I investigated was the globe artichoke. Artichokes are grown almost exclusively in coastal California as perennials where cool summers and frost-free winters allow year-round production.

We have learned to manipulate the growth of the artichoke to produce edible flower buds the first year. To induce the artichoke plant (a biological biennial) to produce buds the first year, germinating seedlings must be given a cool moist treatment (vernalization).

In early March, germinated seeds were planted in 1-quart containers in a greenhouse maintained at 50-75 F to prevent devernalization.

In early April the plants were moved to a cold frame for hardening. Sash covers were only used on the threat of frost. In early May the plants containing six to eight leaves were planted 2 feet apart in rows 4 feet apart. To maintain cool soil and control weeds, the plants were mulched with hay or undecomposed leaves.

Harvesting began about July 1 and continued until mid-August. Plants that failed to produce buds by July 10 were

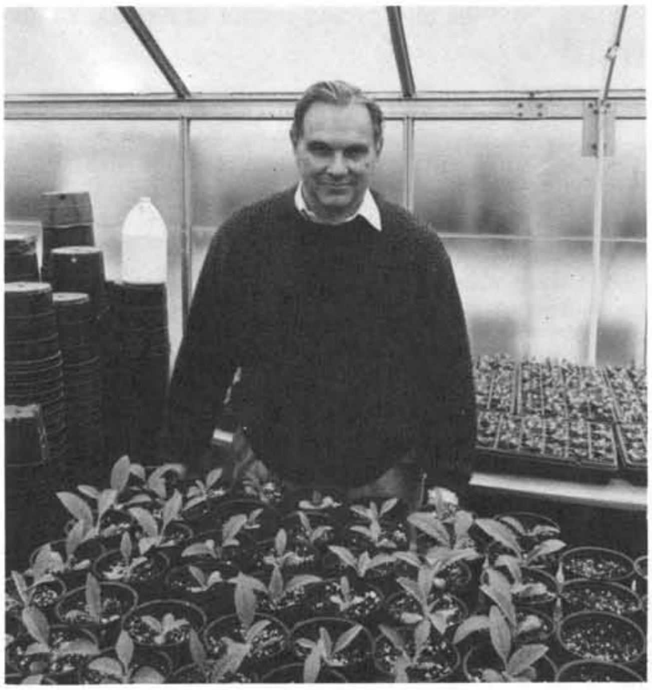

**Figure 1. David E. Hill and artichoke plants growing for spring transplant.**

treated with 100 parts per million gibberellic acid, a natural plant hormone, to induce bud formation. The treated plants produced buds from mid-August until killing frosts in late October or early November. The average yield/plant ranged from 2.5 to 4.5 buds/plant or 13,600 to 24,500 buds/acre.

Plants that produced buds in early July died back after the last bud was harvested, and new sprouts emerged from the base of the plant. About 5% of these plants produced a second crop in late-September.

During the course of the study, 30 cultivars of artichokes from France, Italy, Spain, Greece, and Israel were tested. Only Green Globe from California and Grande Beurre from France consistently approached California yields.

*Radicchio*

Radicchio (pronounced rah-deek-yo), a chicory, is another niche crop that I investigated during 1984-1987. This lettuce-like crop has been long grown in the Venetto region of Italy where most imports originate. The greenish bronze plant has a dense heart with maroon leaves and creamy white veins.

Radicchio is becoming increasingly popular because it adds color and a tangy taste to salads. It is relatively easy to grow, yet it commands a high price in the marketplace. Unlike its relative witloof chicory (Belgian endive), which must be forced, radicchio is field grown for direct harvest. For fall harvest, seeds were planted in the field in mid-June in rows 1.5 feet apart. The rows were thinned to 1 foot between plants. If planted in May, the cool soil caused up to 40% of the plants to bolt or form loose heads. In an attempt to produce a spring crop, I set hardened transplants in early May (seeded in a greenhouse March 20). The spring-grown heads were slightly larger than fall-grown heads, but only 75% of the spring-grown plants produced heads compared to 87% of the fall plants. Once the spring plants reached maturity, they had to be harvested within 10 days or they would bolt. Fall-grown plants produced heads over a 6-week period and seldom bolted.

Among the 27 cultivars tested, several were noteworthy. Augusto, Cesare, Inca, Marina, and Red Devil produced over 3 tons/A with 85% of the plants producing marketable heads. Crosara, a variegated pale green cultivar flecked with red, yielded nearly 9 tons/A. Crosara's mild-flavored heads averaged 12 oz and were similar in texture and density to large heads of lettuce. In virtually all trials, the loamy soils at Mt. Carmel produced greater yields than the sandy soils at Windsor, indicating the need for soils with higher water-holding capacities.

*Chinese Vegetables*

Sales of Chinese vegetables have increased during the last decade. Chinese cabbage and Pak choi, which are important staples in oriental cuisine and stir-fry cooking, have become increasingly popular. Chinese cabbage forms a compact barrel-shaped (Napa type) to elongated head (Michihli type) with light green crinkled leaves and broad white midribs. Pak choi forms a rosette of dark green leaves borne on light green to white petioles. Both varieties are low in calories and have high nutritional value with substantial amounts of vitamins A and B and calcium.

Forty-one cultivars were grown as spring and fall crops at Windsor and Mt. Carmel during 1988-1990. All plantings were seeded in a greenhouse about 4 weeks before field transplanting in late-April to mid-May (spring crops) or mid-July to September (fall crops). Nutrient requirements are relatively high and 1000 lb/A 10-10-10 fertilizer was applied at transplanting and 90 lbs/A of ammonium nitrate was side-dressed 3 weeks after transplanting. In Windsor's sandy soil, severe leaching by heavy rains caused cracking of petioles, a symptom of boron deficiency.

Uniformity of maturity of cultivars of Chinese cabbage and Pak choi creates a short harvest span. Cold soil and/or increasing day length in late spring can cause premature bolting. For most cultivars the planting window in spring was less than 2 weeks (May 1-10). The window expanded to 3 weeks for Two Seasons (April 20-May 10) and China Flash and Nerva (May 1-20). Blues and Kasumi were planted April 20-May 20 without the danger of excessive bolting.

The Pak choi cultivars Joi Choi and What-A-Joy Choi provided satisfactory yields and quality in plantings from April 20-May 20. Spring-grown heads matured quickly and had to be harvested within a span of a week to prevent bolting.

For fall harvested crops, mid-July plantings of some cultivars suffered losses from head rot, but losses for Blues, China Pride, Dynasty, and Nerva were few. Losses from soft rot in August 1-15 plantings reached 20-25%, but losses in late August plantings seldom reached 8%. Losses in the Pak choi cultivars Joi Choi and What-A-Joy Choi in August plantings were less than 10%.

Most cultivars of Chinese cabbage and Pak choi in fall plantings held well in the field for 2-3 weeks because temperatures were cooling and day length was decreasing. Yields of spring-grown China Flash, China Express, Nerva, and Spring Triumph exceeded 17.5 tons/A. Yields of fall-grown Chorus and Magica exceeded 17 tons/A and Dynasty exceeded 22.6 tons/A. Pak choi cultivars of Joi Choi and What-A-Joy Choi provided the best yield and quality in spring and fall plantings. Mei Qing Choi, a miniature cultivar highly prized for its taste and quality, yielded 7.5 tons/A.

*Spanish Onions*

The most recent crop I have investigated is Spanish onions. Onions have been grown in Connecticut since colonial days. In fact, selections by Connecticut growers in the 1800s provided several commercial types, such as Southport Yellow, Red Globes and Wethersfield Red, which survive today. The increased need for Spanish onions for fast-food and other restaurants has created a price advantage, especially in the late-summer and early-fall markets, before western-grown onions reach the markets in October. The market price, especially for white and red Spanish onions, remains high throughout the winter because storage durability is relatively short compared to yellow Spanish or smaller cooking onions.

Spanish onions must be grown from transplants, to allow sufficient time to form large bulbs for late-August harvest. Accordingly, onions were seeded in the greenhouse in early March for transplanting in late April. Ample nitrogen and water were supplied to the forming bulbs.

In 1990-1991, up to 10 Spanish types were tested at Windsor and Mt. Carmel. In 1990, an exceptional year for growing onions in the Northeast, yields of Ringmaker and Riverside exceeded 1 thousand 50 lb bags/acre at Windsor compared to a national average of 720 bags/acre. In 1991, Gringo yielded 930 and Hybrid Big Mac yielded 800 bags/acre at Windsor. These cultivars were durable in storage up to 4 months. The yields of all cultivars at Windsor on a sandy soil were 4 to 6-fold greater than at Mt. Carmel on a heavier loamy soil.

The niche crops that I have studied over the past 8 years have provided opportunities for many growers who have either grown these crops for the first time or who have exchanged their old cultivars for newer ones with improved yield and quality. Many who operate roadside stands have found that these new crops can be priced independently of foreign and western imports.

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# Anthracnose fruit rot of strawberry found in Connecticut fields

**By James A. LaMondia**

In early June 1991, an anthracnose fruit rot of strawberry appeared in Connecticut. While new to Connecticut, this disease is extremely important and common in the South. Warm weather and humidity favor spore production, germination and infection by the anthracnose-causing *Colletotrichum* fungi, so the unexpected outbreak may be related to the unusually warm weather during the spring of 1991.

Symptoms included circular, sunken, water-soaked tan to brown lesions on both green and ripe fruit (Fig. 2). Under wet or humid conditions, creamy pink to salmon colored spore masses occurred at the centers of these lesions, and the fungus produced fluffy white growth at the border of the lesion and healthy tissue. Lesions continued to expand under dry conditions and, as long as secondary pathogens did not cause soft rots, the fruit became mummified and black. Disease was widespread over at least three counties, and severity ranged from light infections to a 100% loss. One 2-acre planting in Tolland County was destroyed.

I repeatedly isolated *Colletotrichum* fungi from fruit, petioles, runner stolons, and fruit trusses but not from leaf tissue in New Haven, Hartford and Tolland Counties. These isolates varied considerably, ranging from gray to bright rose-colored in culture. Healthy green strawberry fruit inoculated with fungi from culture resulted in similar symptoms after 3 to 4 days, and the pathogen was re-isolated from diseased fruit.

At least three species of *Colletotrichum* fungi, *C. acutatum*, *C. fragariae*, and *C. gloeosporoides*, cause anthracnose fruit rots in the South. One of these species can also cause a severe crown rot that can kill entire plants. Because the three *Colletotrichum* species differ in pathogenicity, or ability to cause anthracnose, it is important to know which species caused the outbreak of disease in Connecticut. *C. fragariae* can cause more severe petiole and crown rot symptoms than *C. acutatum*. In turn, *C. acutatum* causes more severe symptoms than *C. gloeosporoides*. Additionally *C. fragariae* and *C. gloeosporoides* have been shown to cause leaf lesions, while *C. acutatum* does not.

Connecticut isolates were identified as *C. acutatum*. Their fungal spores (conidia) were pointed on at least one end, lacked setae (hair-like structures), and had colony diameters ranging from 4.0 to 5.0 cm after 5 days at 25 C. The other two species have cylindrical spores, produce setae, and have colony diameters of about 7.0 cm under the same conditions.

These taxonomic results were consistent with my observa-

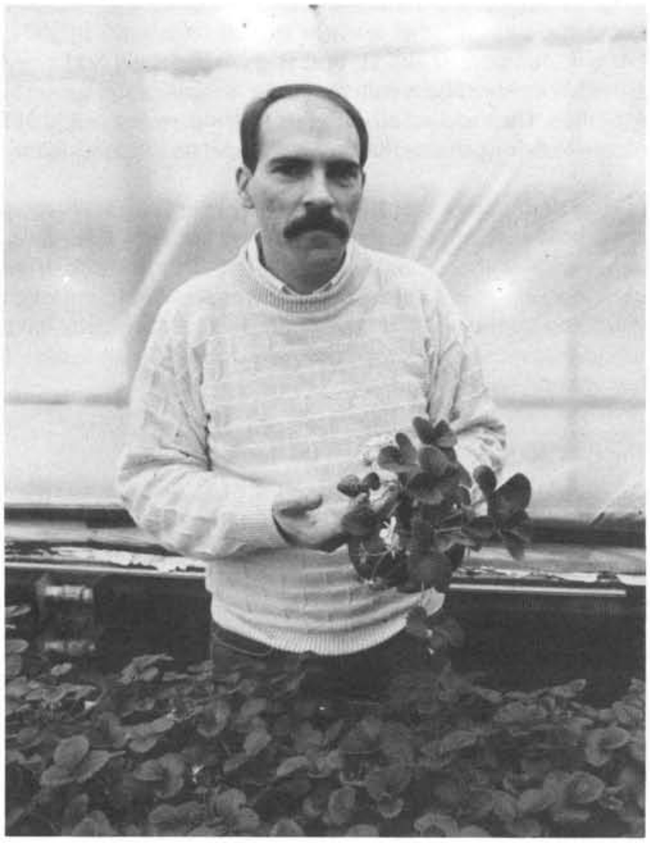

Figure 1. James A. LaMondia with strawberries in a greenhouse at the Valley Laboratory in Windsor.

tion that leaf infection and crown rot did not occur, even in fields with severe fruit rot problems and high inoculum levels of this fungus.

Anthracnose fungi overwinter in plant debris such as mummified fruit, or in lesions on runner stolons or leaf petioles. In the spring, fungi produce conidia which may be dispersed by water splash, or wind driven rain, and by people or equipment moving through the field. Under humid conditions conidia germinate and infect new plants. Lesion expansion and new conidia production occur more rapidly as temperatures increase. A few rainy days during warm weather can quickly spread and increase the pathogen, initiating a fruit rot epidemic.

Control of anthracnose fruit rot has proven difficult in the South. The use of disease-free crowns as planting stock and crop rotation help to reduce initial infection and disease within a crop. The manipulation of moisture and humidity by thinning plants and controlling weeds to increase air flow through the canopy, or reducing overhead irrigation, can slow the spread and rate of increase of the disease. Additionally, a number of fungicides can limit infection and protect the fruit.

I tested the effectiveness of captan, benomyl, vinclozolin, and thiram fungicides to control this fungus in the laboratory. Tests were performed by adding known concentrations of each compound to potato dextrose agar growth medium and placing plugs of agar infested with the fungus in the center of petri dishes. The diameter of the fungal colonies was measured periodically to determine the reduction in growth rate and effectiveness of the fungicide. The Connecticut isolates of *C. acutatum* were sensitive to benomyl at low concentrations (0.5 ppm). Other fungicides such as thiram and vinclozolin also inhibited fungal growth, but only at higher concentrations (Table 1).

**Table 1. Colony diameter of *Colletotrichum acutatum* grown in potato dextrose agar amended with 0 to 50 ppm of active ingredient (a.i.) fungicide. Colony diameter in the absence of any fungicide was 6.5 cm.**

| | Colony diameter (cm) after 5 days at 24 C | | | |
|---|---|---|---|---|
| | Fungicide | | | |
| Rate a.i.(ppm) | Captan | Benomyl | Vinclozolin | Thiram |
| 0.5 | 6.3a | 3.4a | 6.7a | 6.4a |
| 1.0 | 6.3a | 2.9b | 6.6a | 6.3a |
| 5.0 | 6.5a | 2.7c | 5.8b | 4.9b |
| 10.0 | 6.2a | 2.7c | 4.9c | 5.0b |
| 50.0 | 5.3b | 2.6c | 3.6d | 2.9c |

Letters within columns followed by the same letter not significantly different (LSD: $p = 0.05$).

The overwinter survival of the anthracnose fungus in crop debris and dormant plant infections will be investigated this spring. A combination of control tactics such as crop rotation out of fields with anthracnose fruit rot and replanting with disease free crowns, the manipulation of conditions to reduce moisture and humidity to the extent possible, and the appropriate use of fungicides under conditions suitable for anthracnose, will be evaluated as means of anthracnose control.

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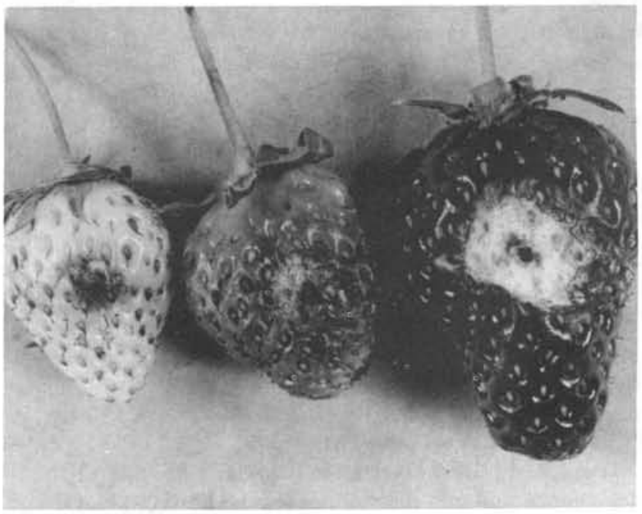

Figure 2. Anthracnose fruit rot of strawberry on green (left), partially-ripe (center), and ripe fruit (right).

# Two species of parasitic mites infest honey bees in Connecticut

**By Carol R. Lemmon**

Two mite parasites recently have become serious pests of honey bees in Connecticut. The tracheal mite, *Acarapis woodi*, is an internal parasite, and the *Varroa* mite, *Varroa jacobsoni*, is an external parasite of the honey bee.

Tracheal mites were first detected in the United States in July 1984 in Texas. They spread rapidly due to the mobility of beekeeping in the United States.

Despite a honey bee tracheal mite quarantine that was established in Connecticut in March 1986, infested honey bees were found in the towns of Woodbury and Watertown in November 1989. The honey bees had been purchased by mail from Georgia in April 1989 by an unregistered beekeeper. This infestation was controlled. We also surveyed a 3 mile area around the infested hives to determine if the tracheal mites had spread via drone (male) honey bees. No other infestations were found.

Two additional apiaries (bee yards) were found to be infested with tracheal mites in January 1990. One colony died; mites in the other bee yard were controlled.

Tracheal mites were subsequently detected in 10 towns in 1990 and have now spread statewide, with at least 28 towns known to have honey bees infested with these mites (Fig. 1). The current infestation is widespread, and may have extended to the wild honey bee population.

The entire life cycle of the tracheal mite takes place in the respiratory system (tracheae) of adult honey bees (Fig. 2) and can be completed in about 10 to 12 days. The pearly white microscopic female mite lays 6 to 9 eggs which hatch in 4 days, and all stages,(i.e., egg, larvae, pre-adults and adults) may be present in tracheae of infested honey bees. The mites have mouthparts that pierce the membrane of the trachea allowing them to feed on the hemolymph (blood) of the honey bee. I have found as many as 100 mites in all stages of development in a trachea of a honey bee. Male mites mate with females in the tracheae. Mated females crawl out of the tracheae of the host honey bee to its hair tips and transfer to young adult honey bees that are less than 4 days old. Mated females enter the trachea of the young adult honey bee via a spiracle, an outside opening of a breathing tube. The population of mites reaches its peak in the winter, the best time for diagnosis and surveillance, and is lowest in the summer.

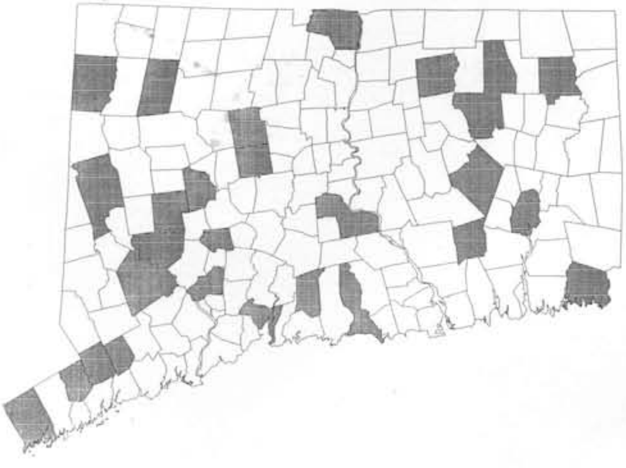

**Figure 1. The shaded areas show towns where tracheal mites have been found in Connecticut.**

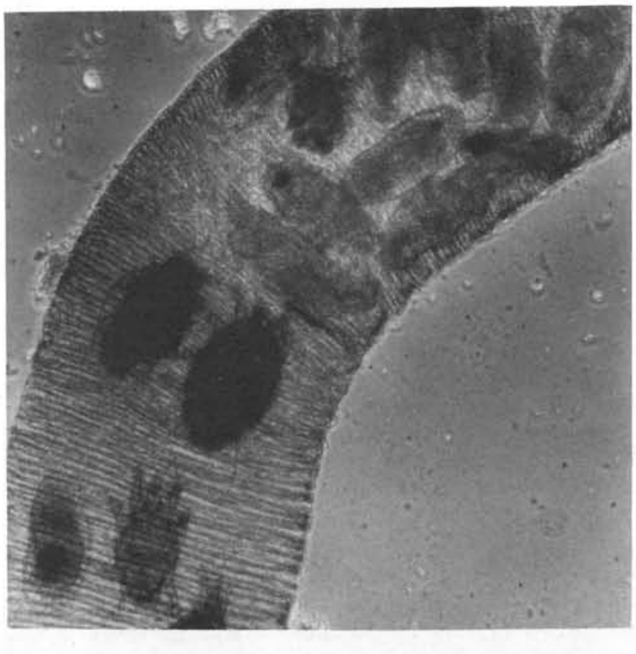

**Figure 2. Eggs and immature larvae of the tracheal mite, *Acarapis woodi*, feeding in the respiratory tube of a honey bee. Enlarged 242 times. USDA photo.**

Tracheal mites can clog the respiratory tubes of the honey bee and reduce its oxygen uptake. Symptoms of tracheal mite infestation include crawling bees near the front entrance of the hive with *K-wing*, in which two wings on one side of the bee separate, or small clusters of dying bees in the winter, even when there is plenty of stored honey available to nourish them.

Menthol is the only material registered for control of tracheal mites. It vaporizes at temperatures above 65 F and is put in the hive for 14 days in the early fall just after the honey for human consumption is removed from the hive. Menthol fumes kill all stages of the tracheal mite except the egg, but this control method is effective only when infestations are low.

*Varroa* mites were first discovered in the United States in a Wisconsin apiary in September 1987; they were first reported in a Newtown, Connecticut bee yard during November 1991. We conducted a survey of 60 apiaries in widely separated sites in Connecticut during the fall of 1991 and found

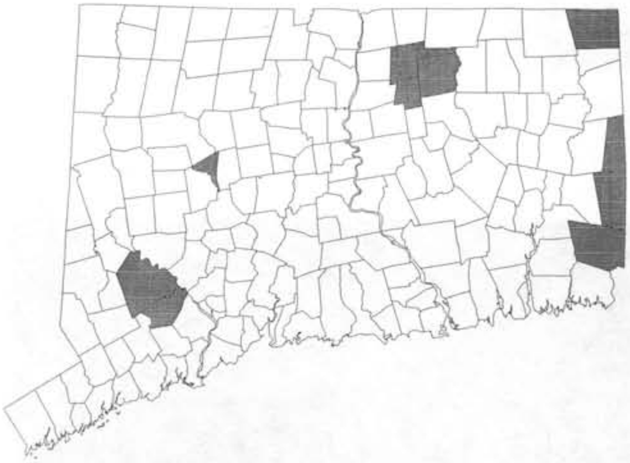

**Figure 3. The shaded areas show towns where *Varroa* mites have been found in Connecticut.**

*Varroa* mites in 64 hives owned by 21 beekeepers in 10 towns (Fig. 3).

The female is reddish brown and can be seen with the naked eye as it is about the size of a pinhead. She feeds on hemolymph of adult honey bees as well as immature bees. The female crawls into a cell containing the immature honey bee and lays eggs at varying intervals within the confines of the capped cell. Feeding by immature mites often causes a high degree of deformity, such as misshaped wings and legs, and usually results in premature death.

The male mites mate with females within the cell. The mated females then leave the cell and seek a blood meal from a passing honey bee before entering another cell about to be capped.

*Varroa* mites can be eradicated or suppressed by a chemical pesticide called fluvalinate. This product is impregnated in plastic strips that can be hung in bee hives after all the honey for human consumption has been removed. Honey bees that come in contact with the strip are not harmed, but mites clinging to their bodies are killed. *Varroa* mites are not protected by the capped cells during the winter, and in some instances, we have greatly reduced or eradicated heavy infestations of these mites using the fluvalinate strips.

An alternative solution for mite control is the use of resistant strains of honey bees. For example, there is evidence that some breeds of honey bees may be resistant to tracheal mites. Cross breeding and testing are being conducted across the country to confirm these findings.

To clarify the extent of tracheal and *Varroa* mite infestations in Connecticut, I will be examining feral (wild) honey bees this summer to determine if they are parasitized by either of these mites. This information will be particularly helpful in our assessment of the effectiveness of *Varroa* mite quarantines.

*Xavier Asbridge, Dora Brown, Stephen Sandrey and Peter Trenchard assisted with inspections and treatments*

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