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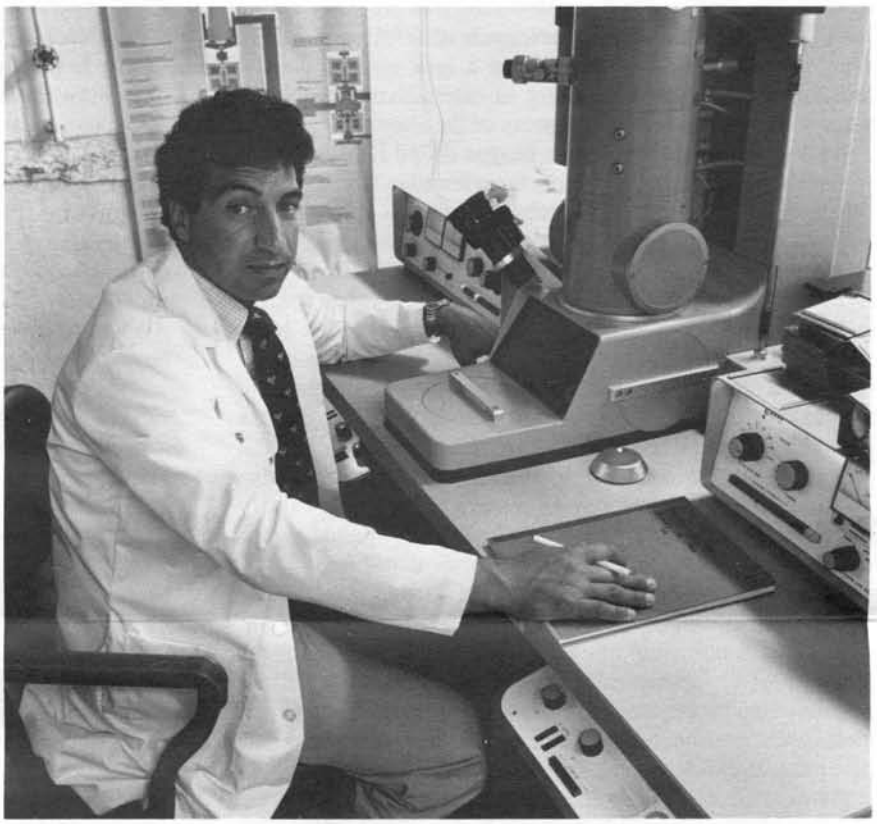

Theodore G. Andreadis in electron microscope laboratory

Fungus causes gypsy moth mortality

Salt suppresses asparagus disease

Instruments measure environmental contaminants

Insecticides near home reduces tick risk.

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION, founded in 1875, is the first experiment station in America. It is chartered by the General Assembly as an independent State agency governed by a Board of Control. Station scientists make inquiries and experiments regarding plants and their pests, insects, soil and water quality, food safety, and perform analyses for State agencies. Factual information relating to the environment and agriculture is provided freely and objectively to all. The laboratories of the Station are in New Haven and Windsor; its Lockwood Farm is in Hamden. Copies of this and other publications are available upon request to Publications; Box 1106; New Haven, Connecticut 06504

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Fungus from Japan causes heavy mortality of gypsy moth

By Theodore G. Andreadis and Ronald M. Weseloh

In the spring of 1989, heavy gypsy moth, *Lymantria dispar,* infestations were detected throughout much of Fairfield County, and significant defoliation was predicted as we anticipated the beginning of a new cycle of defoliation. However, in early June, we discovered a new natural enemy causing unprecedented mortality in caterpillars throughout many forested and residential areas of the state.

This new natural enemy is a fungus called *Entomophaga maimaiga* and it had never before been found in North American gypsy moths. The fungus probably originated from Japan, and we believe it may be identical to one that was imported and released at several locales near Boston, MA in 1910-11. This Japanese "gypsy fungus," as it was called, was thought to have failed to have become established and was never seen again until 1989. Although we can only speculate, this fungus could have survived and remained undetected until 1989 when the unusually wet weather and large susceptible gypsy moth population collectively created an ideal condition for the fungus to express itself in epizootic proportion. It is also possible that the fungus may have been unknowingly introduced from Japan at another time via contaminated gypsy moth egg masses which can harbor dormant stages.

The manner in which *E. maimaiga* causes infection in gypsy moth caterpillars is typical of other entomopathogenic fungi. The usual route of entry is through the integument (outer skin) via spores called conidia (Fig. 1). When these conidia come in contact with the soft integument of the gypsy moth, they germinate and send out an invasive tube that penetrates the body cavity. Inside the body, the fungus grows at the expense of the insect's softer tissues. It multiplies by budding and fission until the insect's body cavity is filled with many irregularly shaped structures called hyphal bodies.

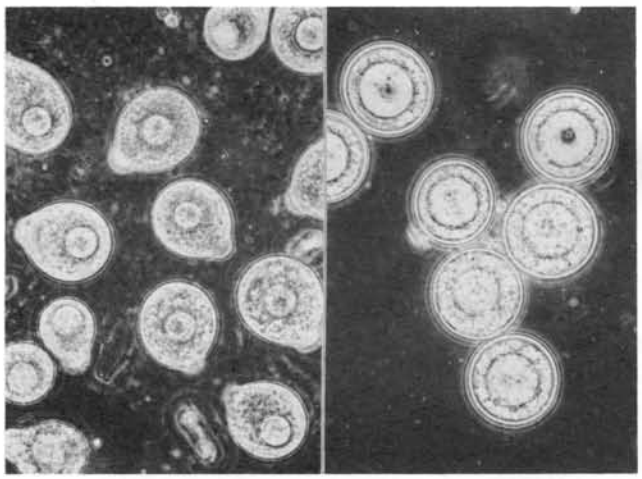

Figure 1. Conidia (left) and resting spore (right) stages of *Entomophaga maimaiga* (x300).

Infected caterpillars show reduced vigor and eventually die, typically clinging to the trunk of the tree with their heads facing downward. Caterpillars infected in the laboratory usually die within 7-10 days but the process probably takes longer in nature. In the presence of high moisture, the fungus will then grow outward through the body wall and form new conidia on sporebearing structures called conidiophores. Infected cadavers appear greyish-green owing to the external growth of the fungus. Conidia are forcibly discharged into the surrounding air, and when they come into contact with another caterpillar, they adhere and produce an invasive tube that enters the body and begins a new cycle of infection. If moisture is not adequate for conidial development, the fungus will remain internal and produce large spherical resting spores (Fig. 1). Resting spores have thick walls and are highly resistant to adverse conditions, such as freezing and drought, that would ordinarily destroy the conidia. It is within this stage that the fungus is believed to overwinter and remain dormant until conditions are right for germination and subsequent infection of caterpillars in the spring.

We found fungus-infected caterpillars in virtually all towns where gypsy moths were present (108 towns in 6 counties) (Fig. 2). The highest prevalence of the disease was in Fairfield County where the largest gypsy moth infestations were present. However, the fungus was also detected in many of the outlying towns where gypsy moth populations were barely noticeable, including parts of Middlesex, Tolland and Hartford Counties. Infected caterpillars were also found in Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, and Vermont indicating that the fungus is widely distributed in the Northeast and has probably been here for some time.

Most of the fungal-induced mortality occurred in late June and early July as caterpillars were developing to the 4th and 5th stages. As a result, defoliation was primarily limited to the oaks, and many less preferred trees (i.e. beech, birch, and maple) that would normally have been defoliated were scarcely attacked. A total of 164,338 acres of forested land, mostly in Fairfield County (Fig. 2), experienced some defoliation in 1989. However, only 40,185 acres were severely defoliated (greater than 75%) and more than half of the acreage (85,908 acres) was defoliated less than 25%, which is barely noticeable. We feel that had the fungus not been present, our forests would probably have sustained far more severe defoliation as in 1980, when comparable gypsy moth populations defoliated nearly 400,000 acres. Significant declines in egg mass densities were also observed in many infested areas of southwestern Connecticut (especially Fairfield County), and contrary to the last outbreak in 1980-83, there was little eastward expansion of the infestation. However, the population did not collapse everywhere and increased egg mass densities were recorded along the leading edge of the infestation (Litchfield, Hartford, and New Haven Counties).

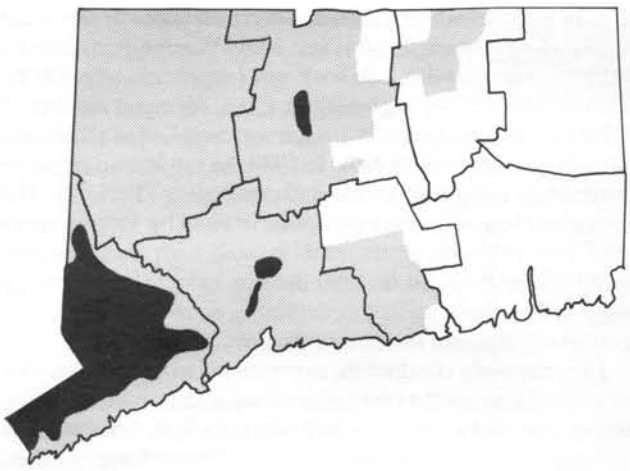

Figure 2. County map showing areas of the state that were defoliated 10% or more by gypsy moths in 1989 (black) and the distribution of fungal-infected caterpillars (shaded).

We can only speculate on the long-range impact that the fungus may have on gypsy moth populations in the future. The fungus now appears to be firmly established throughout the Northeast and there is probably more fungal inocula in the environment than at any other time. However, given its apparent dependence on moisture in the form of rainfall, the degree to which the fungus will suppress gypsy moth populations and help prevent outbreaks in the future is unknown. The above average rainfall in the spring of 1989 (17 inches on 24 different days in May and June) played a major role in allowing the fungus to germinate and infect caterpillars. If there is another wet spring in 1990 we would expect similar mortality again.

We have isolated the fungus from caterpillars collected from several different towns and are maintaining it in an artificial insect cell medium in the laboratory. We are planning field studies for 1990 and are also exploring ways in which we might use the fungus as a biologic agent to help manage gypsy moth populations in the future. We are strongly encouraged by the fact that the fungus grows readily in culture, does not pose any health hazards to animals or the environment, and can cause widespread disease in rising gypsy moth populations.

Although we are certain the gypsy moth will never be eradicated, this newly discovered fungus may prove to be an effective weapon in helping to prevent the large periodic outbreaks of gypsy moth that plague us every 8-10 years.

Rock salt helps suppress crown rot of asparagus

By Wade H. Elmer

Fifty years or more ago asparagus growers could expect 15-20 years of productivity from an asparagus field. Beginning in the 1940's, crown and root rot began killing large numbers of asparagus plants; this same disease is the main factor limiting production today. The disease is caused by two similar soil-inhabiting fungi, *Fusarium oxysporum* and *F. moniliforme*. These fungi are common to most soils and act in concert to slow growth and reduce productivity. The fungi infect and colonize the roots, crowns, and stems and cause the ferns to turn yellow, wilt, and die. Eventually, the crown collapses and the entire plant succumbs. This disease can destroy over half the plants in a field in less than 5 years, which is when a planting should become most productive. Furthermore, the fungi persist in the soil for long periods of time, preventing old asparagus fields from being replanted.

It was not known why the disease suddenly appeared in the 1940's. Asparagus had been grown in the Northeast since the early 1900's, and the fungi causing the decline have been present in the Northeast even longer. Because no new cultivars had been introduced, I began searching for changes in cultural practices that might explain the advent of the disease. I learned that rock salt, an unrefined form of sodium chloride, was once applied nearly universally on asparagus beds in the early days of its cultivation in this country. Asparagus thrived in saline marshes in Europe, and late 19th Century gardening books suggested liberal application of salt. Although there was no clear consensus on rates or timing of applications, the use of rock salt in asparagus culture was supported by research findings and became a reputable cultural practice in the early 1900's.

Although the benefits of salt were well documented, its mode of action is a mystery. Some speculated that rock salt improved asparagus yields mainly because it suppressed and killed competing weeds, but research documented increased asparagus yields in the presence of salt but in the absence of weeds. Other mechanisms seemed to be involved. Despite the incomplete understanding of the effect of salt on asparagus, the tradition continued into the 1940's.

As growers began using synthetic herbicides, the use of salt was eliminated. Strikingly, the first reports of Fusarium crown and root rot were almost coincidental with the discontinuation of application of rock salt.

I hypothesized that the chloride component of rock salt may have been suppressing Fusarium crown and root rot disease prior to the 1940's. This suspicion is supported by recent studies that demonstrated suppression of other soil-borne plant diseases by different forms of chlorides. I therefore designed experiments to learn if sodium chloride could

Table 1. Yield in pounds of spears per 20 feet of row and colonies of Fusarium spp. per cm of root in a 2-year-old asparagus planting treated with rock salt.

	Spear wt		Colonies
Treatment	1988	1989	1989
Untreated	0.8	2.4	0.56
Salt	0.7	3.2*	0.37*

* values are significantly different from the untreated values at P = 0.05.

suppress Fusarium crown and root rot of asparagus with the ultimate goal of helping growers return asparagus culture to Connecticut.

In a greenhouse I applied salt to plants at the rate of about 0.35 oz per ft^2 and left an equal number of plants untreated. All plants received a complete supply of nutrients. Both treated and untreated plants were grown in sterile soil or in soil that was infested with *F. oxysporum* or *F. moniliforme.* After 2 months of growth, I recorded plant weights and root lengths and assessed the amount of disease and soil fungal densities. The fungal densities in the infested soils were unchanged indicating that salt had no fungicidal effect, but I found that plants treated with salt were larger, had more roots and had less disease than untreated plants. Salt not only improved plant growth in the absence of the fungi, but it increased the resistance of the asparagus root tissue to infection. For example, when the young feeder roots were incubated on an agar medium selective for *Fusarium* spp., significantly fewer colonies of *Fusarium* grew from roots of salt-treated plants than from roots from untreated plants. Likewise, the number of root lesions and discolored areas were less on roots from salt-treated plants. These findings suggest that salt increases disease resistance and also may have some growth-enhancing properties.

In 1987 I began several field experiments at Lockwood Farm in Mt. Carmel and at the Valley Laboratory in Windsor to evaluate the effects of salt on yield and disease. In a asparagus (cv. Mary Washington) field planted at the Lockwood Farm in 1986 I established 10 replicated rows that were 6 ft by 5 ft. All plots received ammonium nitrate at 100 lb N/A. On half of the plots I applied 11 oz of rock salt (approximately 1000 lb/acre) in the spring of 1987, 1988 and 1989. The other five plots were left untreated. The planting was picked for 2 weeks in 1988 and for 3 weeks in 1989. No differences in yield were found in 1988, however, yields significantly increased in salt-treated plots the following year (Table 1). Three months after treatment in 1989, I sampled roots from treated and untreated plants and incubated them on selective agar. Fewer colonies of Fusarium spp. grew from roots from salt-treated plants than from untreated plants.

To evaluate the effect of rock salt on yield from older diseased plants, I applied rock salt to 6-year-old asparagus plots in Windsor where yields had declined annually due to the disease. Four asparagus plots (cv. Mary Washington) 20 ft by 5 ft were treated with 1 lb rock salt (approximately 500 lb/acre) for three years beginning in 1987. An equal number of plots were left untreated. Nitrogen was supplied to all plots as calcium nitrate at 100 lb N/A. In 1988 the salt had no effect on yield when compared to the untreated plots (Table 2). The untreated plots continued to decline in yield by 19% between 1988 and 1989. However, plots treated with rock salt produced 8% more yield in 1989 than in 1988. These findings suggest that applying salt to declining asparagus fields may help arrest or retard the rate of decline.

I am currently conducting experiments to understand why rock salt increases the resistance of asparagus to disease. It is known that root exudates which normally leak from actively growing root tips will stimulate these soilborne fungi to infect. It is possible that salt affects the nutritional status of the plant cell, which in turn alters the quantity and/or quality of root exudates.

Assuming that the positive response of asparagus to salt applications that I have seen so far continues, the use of rock salt may then become a new weapon in our arsenal to suppress Fusarium crown and root rot of asparagus. A return to the forgotten tradition of applying salt on asparagus fields may help to restore asparagus culture to Connecticut.

Table 2. Yield in pounds of spears per 20 feet of row in a 6-year-old asparagus planting treated with rock salt.

Treatment	1988	1989
Untreated	1.1	0.9
Salt	1.2	1.3*

* values are significantly different from the untreated values at P = 0.05.

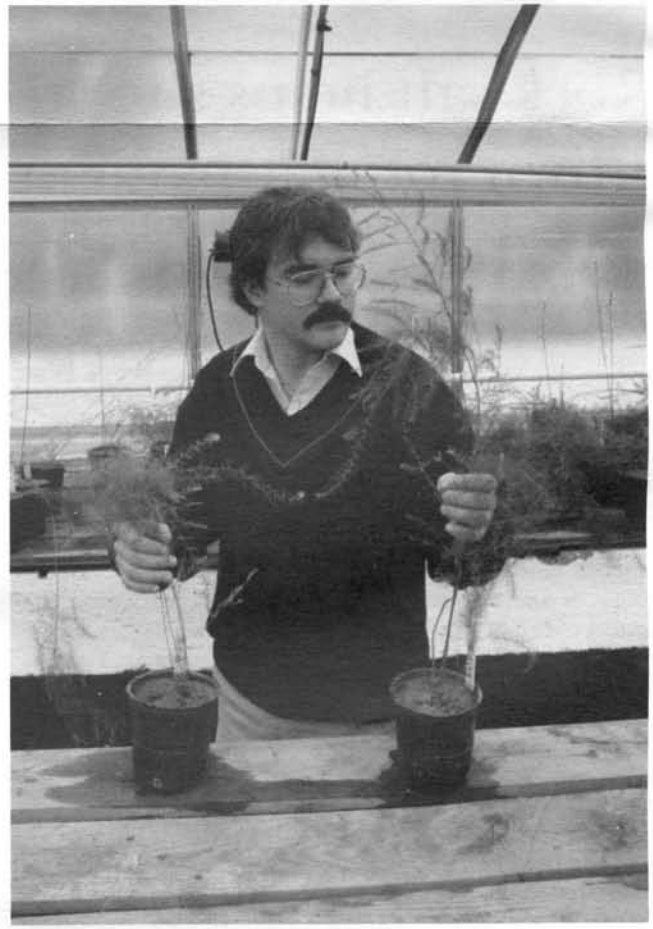

Figure 1. Wade Elmer with asparagus plants growing in a greenhouse.

State-of-art analytical instruments measure environmental contaminants

By Mary Jane Incorvia Mattina

The determination of pesticides in ground water, dioxin in air, and PCBs in fish are but a few examples of the types of analyses undertaken by scientists in the Department of Analytical Chemistry in response both to the public's concerns and to the regulatory demands of the State of Connecticut. The challenge to the analytical chemist comes from the identification of contaminants previously undetected, as well as from the determination of contaminants at the parts per billion, parts per trillion, and even the parts per quadrillion level. State-of-the-art instrumentation is required to accomplish these qualitative and quantitative goals.

Our most impressive new instrument is a high resolution, magnetic sector mass spectrometer for the detection of dioxins and furans in environmental samples. Because of the high toxicity of these compounds, the analytical method developed by the Federal Environmental Protection Agency (EPA) requires instrumentation capable of detecting as little as 500 parts per quadrillion of a particular dioxin or furan. To get some idea of what this demands of the instrumentation, consider the following problem. Suppose we cover the following states with a single layer of blocks, each of which is 1 inch on an edge: Vermont, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Maryland, Virginia, North Carolina, South Carolina, and Georgia. It would take 1.0642 x 10^{+15} blocks, or 1,064,200,000,000,000 blocks to accomplish this task. Now suppose that all the blocks are black except for 532 blocks which have been painted red and are scattered randomly throughout the area covered. The problem is to find the 532 red blocks. The instrument has about 10 seconds in which to do the job. This is a formidable task demanding the extraordinary sensitivity of magnetic sector mass spectrometers.

Figure 1. Mary Jane Incorvia Mattina with the high resolution, magnetic sector mass spectrometer.

Sensitivity is but one of the demands of the EPA method for the measurement of dioxin and furan that we are implementing in our laboratories. The method also requires that the analyst demonstrate that the instrument is responding to dioxin and furan and not to interfering compounds. Interfering compounds can be removed from the sample by means of extraction and cleanup. The extraction is sample specific; that is, it depends on whether fish, soil, air, incinerator fly ash, milk, etc. is to be tested. For example, if a fish sample is to be analyzed, boiling solvent is continuously drained through a portion of ground up fish. Extraction is followed by four or five cleanup steps, each of which is designed to remove a group of potentially interfering compounds, which may be present. Each step is labor intensive; for example, in one of the cleanup steps four different solvents must be used in a specific order. In addition the analysis requires eleven isotopically labelled compounds for quantitation of the dioxin and furan.

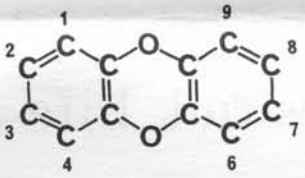

Figure 2. The dioxin molecule.

The analysis is further complicated by the fact that neither the term "dioxin" nor "furan" refers to a single compound. For the dioxin series of compounds the EPA method requires the qualitative and quantitative identification of seven different dioxin congeners and for the furan series the identification of ten different congeners. A dioxin congener is a molecule with a specific arrangement of carbon and oxygen atoms and a variable arrangement of chlorine and hydrogen atoms. Figure 2 illustrates the basic structure of the dioxin molecule. A chlorine atom may bond to the carbon atom at each position

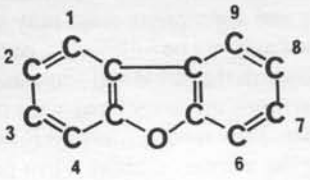

Figure 3. The furan molecule.

labelled 1-4 and 6-9. The dioxin molecule, therefore, may have as few as one or as many as eight chlorine atoms, and there are several different arrangements of the chlorines on the basic dioxin structure. Any carbon not attached to a chlorine has a hydrogen atom bonded to it.

Congeners are also possible for furan, but for this molecule the basic structure is as shown in Fig. 3. Once again a chlorine atom may bond to any or all of the carbon atoms at positions 1-4 and 6-9, leading to several different furan congeners.

The EPA method is to be followed for instrumentation, sample workup, and data reduction if the data generated are to be reliable. Once dioxins and furans have been reliably analyzed according to EPA protocols, we can proceed to a longer range goal of designing and carrying out dioxin-related research projects based on monitoring the Connecticut environment in collaboration with other state agencies.

To supplement the Station's capability to perform dioxin analyses, a second mass spectrometer has been installed. Known as an ion trap detector, this bench top mass spectrometer is less complicated to operate and maintain than the magnetic sector mass spectrometer. The ion trap detector is meant to complement, but not replace, the sector machine. While the sensitivity of the ion trap detector is reputed to exceed that of other bench top mass spectrometers, we intend to investigate precisely the minimum dioxin detection limits on this machine. Additional projects are planned to determine if congener specificity can be achieved using the EPA extraction and cleanup methods followed by analysis on the ion trap.

As implied in the discussion above, mass spectrometers can detect extremely small amounts of compounds. They do this with minimal interference from false positives, making detection by mass spectrometry very desirable. For the identification of environmental contaminants mass spectrometers are frequently attached to gas chromatographs. Environmental samples are often mixtures of compounds and the gas chromatograph separates the mixture into its individual components. This procedure works well for a number of pollutants, but most environmental contaminants are not amenable to gas chromatography.

To overcome this obstacle, the Station recently purchased a particle beam interface to couple a liquid chromatograph to a mass spectrometer. This makes it possible to screen environmental samples for contaminants which previously were inaccessible to detection by mass spectrometry. Using the particle beam interface, we have developed methods for the detection of pesticides such as the urea herbicides diuron, linuron, and terbacil and the carbamate pesticide oxamyl. In addition we have expanded the capability of one of our mass spectrometers to detect negative ions. This increases the sensitivity of the instrument for the detection of many environmental pollutants such as the triazine herbicides.

These instruments will be used to provide the citizens of Connecticut with answers to questions regarding environmental quality. With these instruments, analytical chemists at the Station can now monitor the air, food, and water, in fact, our total environment, more thoroughly than before.

Insecticides applied near homes reduces tick bite risks

By Kirby C. Stafford III

Lyme disease is an illness of people and animals caused by the spirochete (bacterium), *Borrelia burgdorferi*. This agent is transmitted by the bite of a tick, *Ixodes dammini*. Interest in Lyme disease prevention through tick control has increased along with the number of human cases and public awareness of the disease. Potential tactics include the use of repellents while outdoors, manipulation of tick hosts, modification of the tick's environment, and application of insecticides. An objective of tick control research at The Connecticut Agricultural Experiment Station has been to evaluate insecticides.

For homeowners in areas endemic for Lyme disease, small scale applications of an insecticide to control *I. dammini* nymphs may be a suitable strategy for reducing the chance of tick exposure. The use of alternative methods of protection such as repellents and appropriate dress may not be practical on a daily basis and may not be followed. Control efforts may also be directed towards the larval and adult stages of this tick, but *I. dammini* nymphs are associated with the majority of Lyme disease cases. They readily transmit *B. burgdorferi* and are active during the warmer months when people frequent tick-infested areas. Nymphs become active in May, are most abundant in June, and persist at lower numbers at the end of July and early August. Several insecticides are registered for the control of ticks in or around the home or on lawns. These

Figure 1. Kirby C. Stafford III placing a trap used to catch mice harboring ticks.

Table 1. Mean number of *I. dammini* nymphs collected from each CO_2 trap 6 weeks before and after treatment in untreated plots and in plots sprayed with carbaryl, chlorpyrifos, or diazinon in Lyme and East Haddam, Connecticut, in 1988.

Treatment	Mean number of *I. dammini* nymphs Pretreatment	Posttreatment
Control	2.4	0.3
Carbaryl	2.6	0.0
Chlorpyrifos	0.8	0.0
Diazinon	1.2	0.0

include carbaryl, chlorpyrifos, diazinon, and cyfluthrin. The first three may be purchased at garden centers. Cyfluthrin is a relatively new product registered against insect pests on home lawns and is available only to certified applicators. I initiated experiments to test insecticide sprays against *I. dammini* nymphs in the summer of 1988 and again in the summer of 1989 at sites in Old Lyme, Lyme and East Haddam.

In the 1988 tests, I applied three insecticides (carbaryl, chlorpyrifos, and diazinon) at label rates to 6000 ft^2 plots of mixed brush and woodland in mid-July. There were two treatment plots for each insecticide and five untreated control plots. A backpack mist blower was used to apply the insecticides. Tick populations were monitored with traps (two per plot) baited with dry ice (CO_2) at weekly intervals from June through August. To test the effectiveness of an insecticide application in controlling *I. dammini* nymphs around actual homes, I used one insecticide (carbaryl) to treat 1-acre plots at five residences in the summer of 1989. These sites included a mixture of well-maintained lawns and woodlands near the home. Two other sites received no treatment. The ground application of carbaryl was made on June 2, 1989 with a high pressure hydraulic sprayer at the label rate of 1 to 2 pounds of active ingredient per acre. Tick populations were sampled this time by dragging a flannel cloth over the vegetation at each site. Ticks were collected in late May prior to spraying (pretreatment samples), three days after spraying (June 5), and into the fall. Although the insecticide treatment was directed towards controlling the *I. dammini* nymphs in the summer, I also wanted to determine if the treatment had any impact on larval and adult populations later in the season. The other sampling dates were June 19-20; July 5, 7, and 11; July 24-26; August 7-9; September 1; October 24-25; and November 7.

In the 1988 field plot trials, I did not recover any nymphs from the areas treated with carbaryl, chlorpyrifos, or diazinon during the 6 week period after spraying (Table 1). Although few nymphs were recovered in the untreated plots during the period after the spraying because of the seasonal decline in numbers, the reduction in the sprayed plots was statistically significant. Differences in the effectiveness of the various insecticides requires further study. The insecticide applications appeared to have no effect on the emergence of larval ticks in August from egg masses laid by the females in the spring. I collected a total of 282 and 103 larvae from untreated and chlorpyrifos treated plots, respectively.

In the 1989 trials at residential sites, I found that the ground application of carbaryl reduced populations of nymphal *I. dammini* by 100% at all five treated sites (Fig. 2A). No ticks were found 3 days after spraying in the treated sites, but an average of 17 nymphs were collected at each untreated site.

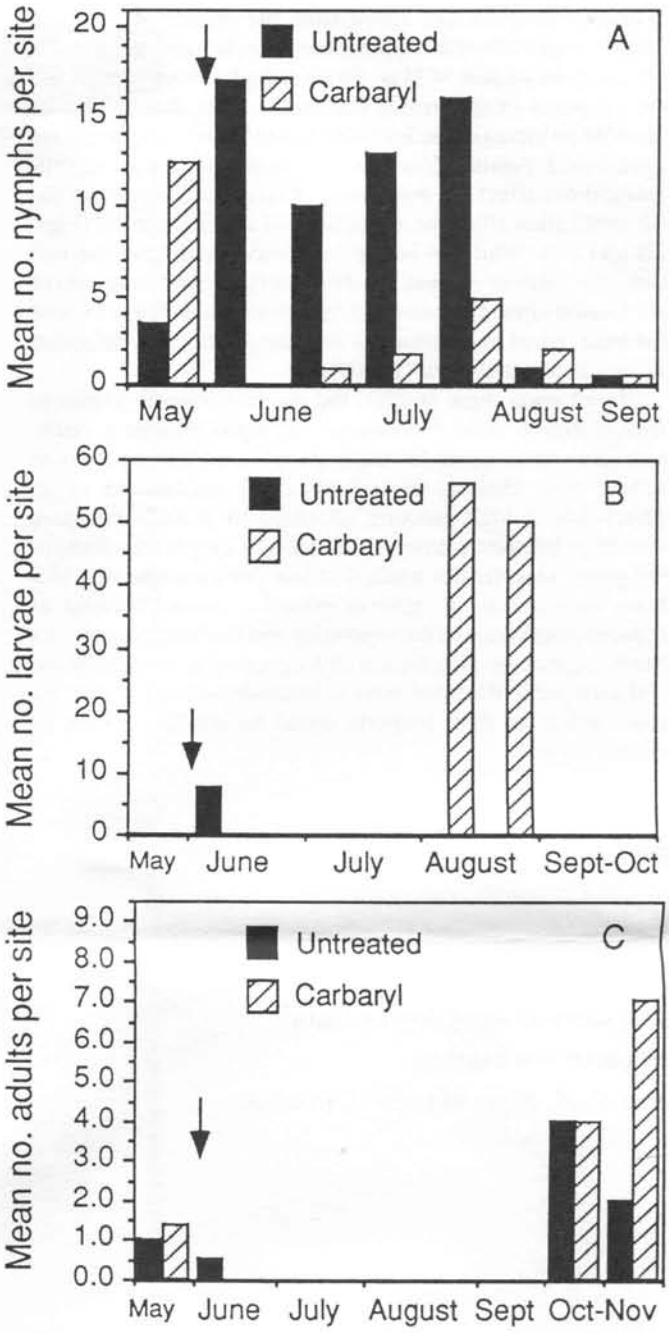

Figure 2. (A) Mean number of *I. dammini* nymphs collected in untreated sites or in areas sprayed with carbaryl in Old Lyme and Lyme, Connecticut, in 1989. (B) Mean number of *I. dammini* larvae collected at the same sites. (C) Mean number of *I. dammini* adults collected at the same sites (only two carbaryl sites were sampled for adults in November). Arrow indicates application of carbaryl.

Although *I. dammini* nymphs were recovered at two of the sprayed plots as early as 2 weeks after treatment and at three of the treated plots by 4 weeks after treatment, I only recovered these ticks at the edges of the sprayed plots in woodlands or tall grassy areas. The treatment appeared to be more effective on the manicured lawns. Nymphs were recovered within the central areas of three of the sites only towards the end of July, 7 weeks after treatment. A second application may be required

to control nymphs still active from the middle of July into August, especially if the application is made a couple of weeks earlier in the middle of May. Since nymphs slowly reinvaded the periphery of the treated residential plots, it may also be possible to increase the level of protection by expanding the treated area. Again, I found that the application of an insecticide did not affect the emergence of larvae in August nor did the application affect the appearance of adults in the fall (Figs. 2B and 2C). While no larvae were recovered from the two untreated sites in August, larvae were recovered from two of the treated sites. Host-seeking larvae are considered to pose the least risk of transmitting *B. burgdorferi* because infection rates are extremely low ($\leq$ 2.0%).

Based upon these studies, the use of currently available insecticides to control *I. dammini* nymphs may be a viable management practice for many people in endemic areas to reduce their chances of tick bite. The application of an insecticide at high pressure appeared to provide effective coverage, but the treatment did not last as long in woodland or tall grass. Insecticides applied at low pressure like that of a hand sprayer may not give as effective control because of reduced penetration of the vegetation and leaf litter. A person's knowledge of the distribution of *I. dammini* around the home and narrowing of treated areas to locations where families are most active on their property could reduce the amount of chemical used.

Figure 3. Mouthparts of an *Ixodes dammini* adult female (center). The backward projecting teeth make it difficult to remove the tick without breaking off the mouthparts and leaving them in the skin.

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