Frontiers of Plant Science

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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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A new mosquito-borne disease caused by West Nile virus was isolated in Connecticut in September 1999, requiring a shift in mosquito surveillance efforts. In the end, new knowledge was gained and the state prepared itself for a possible recurrence in 2000.

Discovery of West Nile virus in Connecticut and what was learned during the first year

By Paul Gough

On Saturday, September 4, 1999, while most people were enjoying the beginning of the Labor Day weekend, Experiment Station Director John F. Anderson was helping a chicken farmer deal with a fly infestation, and became aware, through news media reports, of mosquito-borne illnesses and deaths in New York.

Anderson, an expert on disease-carrying mosquitoes, was puzzled to hear of this outbreak of what was initially identified as the rare St. Louis encephalitis and its extent. He was very aware of such viruses as he and fellow entomologist and Head of the Department of Soil and Water Theodore G. Andreadis were already working on the state-wide surveillance for eastern equine encephalitis and other viruses, including St. Louis encephalitis. He began immediately to plan the Experiment Station's response to the deadly threat near Connecticut's southwestern border.

That evening, Dr. Matt Cartter of the Department of Public Health asked Anderson to participate in a conference call with representatives of the Centers for Disease Control and Prevention (CDC) and New York and New Jersey health officials to learn first-hand what was then known about the virus threat. They learned where people were coming down with the disease, and Anderson took notice of the fact that a few of the suspected cases were in Westchester County, NY, adjacent to Fairfield County. After talking with and getting encouragement from Dr. James Hadler, Connecticut State Epidemiologist, on Sunday morning Anderson prepared six mosquito traps for placement in Greenwich, the closest Connecticut city to the area where the disease was reported.

Because the Experiment Station was already trapping mosquitoes at 37 sites throughout the state for an early warning on viruses, the equipment and expertise were available to increase the number of trapping locations to monitor a possible appearance of St. Louis encephalitis in Connecticut.

Sunday morning Anderson made some dry ice to bait the traps from carbon dioxide gas at the Experiment Station's main laboratories in New Haven and in the afternoon headed for Greenwich.

He first stopped at Nathaniel Wetherill, a home for the aged. He entered the lobby and explained his mission to Louise Comeau who called a supervisor who said a trap could be placed if the Greenwich Police Department approved. After a call to Officer Yasek, the first trap was set. It was raining as Anderson drove to the Greenwich Police Headquarters where Sargent Marr assigned Officer Gleason to guide Anderson to five more Greenwich locations. As the day ended, Anderson had placed the last trap at the Montgomery Pinetum, a place where station entomologist Mark McClure had studied scale insects.

The virus was isolated from two different species at the Experiment Station using the same laboratory and techniques employed in the surveillance for eastern equine encephalitis

As darkness came and the usual overnight period of mosquito activity began, the cone-shaped traps emitted light and carbon dioxide to attract the insects into a cloth mesh enclosure which would prevent their escape.

Labor Day, the next morning, Anderson returned to Greenwich, gathered the six traps, and cooled the captured mosquitoes with ice packs. Tuesday morning, in a laboratory in New Haven, Andreadis and technician John Shepard identified all mosquitoes and separated them into batches of up to 50 of each species for virus testing, which was conducted in another laboratory at the Station by Jodie Correia and Bonnie Hamid.

Also on Tuesday, Anderson called two officials in Greenwich, Caroline Baisley, Health Director, and Larry Cooper, Superintendent of Parks and Trees, to make plans for more trapping. Bob Wagner of the Parks Department was assigned to help Anderson find more likely sites. Anderson also called Phyllis and Paul Mazik, friends of the Experiment Station, who were both retired from the Stamford Health Department, and obtained their help in locating trapping sites in Stamford, the next town from the New York border.

The sites sought were prime mosquito habitat: near marshes and wooded areas where stagnant water allowed mosquito eggs to hatch into larvae and transform into adults in as little as 10 days.

By this time the Connecticut news media were covering the epidemic and the developing response. In a fortuitous circumstance, Tony Campanella, working under Course Superintendent Patrick Lucas of the Innis Arden Golf Club on the Greenwich-Stamford border, who had read one of

these reports, called Anderson about a place on the golf course where mosquitoes were abundant and suggested that a trap could be put there. Anderson, with the aid of Pat Lucas and his staff, placed a trap in the woods near the 4th tee overlooking a pond.

It was there, on September 14, where virus-infected mosquitoes were captured. The virus was isolated from two different species at the Experiment Station using the same laboratory and techniques employed in the surveillance for eastern equine encephalitis. It was tentatively identified as St. Louis encephalitis virus because of its reaction to antiserum to mice infected with St. Louis encephalitis virus and the confirmation by CDC of residents infected with St. Louis encephalitis in New York. These mosquitoes were the only ones to test positive for the virus in Connecticut during 1999.

Although there had never been a reported human case of mosquito-borne encephalitis in Connecticut, a potential killer virus had been found and the public health implications were clear. If that wasn't sufficient to warrant immediate warnings and action, another unusual event was about to be discovered.

In the middle of September reports started to come to health departments and the Experiment Station of an unusual number of dead birds—mainly crows—being found in Fairfield County towns near New York. The first such crow, found on the bank of the Saugatuck River in Westport, was sent for examination to Edward M. Wakem, Richard A. French, Antonio E. Garmendia, and Herbert J. Van Kruiningen at the Pathobiology Department at the University of Connecticut. Its brain was removed and was picked up by Andreadis's technician Colleen Scott, stored in dry ice, and brought it to the Experiment Station for virus testing.

Anderson, who in 1998 had isolated eastern equine encephalitis virus from the brain of a donkey that died in the northeastern Connecticut town of Canterbury, took seven pieces of brain tissue and placed them on Vero cells. Five days later, all seven samples yielded virus. This virus isolate also reacted with antiserum from mice infected with St. Louis encephalitis virus. So now, the virus had been isolated by the Experiment Station from both mosquitoes and a bird and the viruses seemed identical.

At virtually the same time technician Jodie Correia and Shirley Terrell of Yale University confirmed presence of the virus in the crow on September 21, Andreadis was attending a news conference with Governor John G. Rowland and health officials in Greenwich where it was being announced that the Department of Environmental Protection would initiate mosquito control.

Contacted just as the news conference ended, Andreadis informed the Governor's staff and went to the podium to announce the finding to the media. Plans were also made to expand the mosquito trapping in lower Fairfield County.

Two days later, the CDC announced that the virus had been identified as West Nile-like, based on an isolate of the virus made from a bird at the Bronx Zoo, although a definite link had not been made to the illnesses. This finding did, however, clear up questions about the St. Louis virus because it would have been highly unusual for that virus to kill birds.

The Centers for Communicable Diseases and Prevention requested samples of the viruses isolated from the mosquitoes and the crow. The samples, packed in dry ice, were sent from the Experiment Station to the CDC overnight.

On Sunday September 26, Andreadis received a call from the CDC indicating that the virus isolated from the crow and the mosquitoes in Connecticut was identical to the virus identified from a bird at the Bronx Zoo. By September 29, the West Nile-like virus was reported present in the human victims.

With the virus isolated, the research was taking a new direction aimed at finding the exact identity of the virus, determining its action, perhaps even discovering its origin, and looking for the virus in mosquitoes and crows collected elsewhere in Connecticut.

Charles Vossbrinck, working with Anderson and Andreadis, extracted the RNA from the Connecticut virus isolate, converted it to DNA, and with the assistance of the

A potential killer virus had been found and the public health implications were clear

Keck Biotechnology Center at Yale University, the sequence DNA of the virus was determined. Using a database of genes, Vossbrinck searched for similar sequences of DNA. The closest match he found was a strain of West Nile virus from Romania.

West Nile virus is widely distributed in Africa, southwestern Asia and the Mediterranean area, and southern and central Europe. It had not previously been identified in North America.

Library research indicated that West Nile virus could kill birds, but there was no indication that this virus killed large numbers of birds as observed in Connecticut. The reports gave insight into the mosquito species that spread it and the birds that may harbor it. All of this information would be helpful in formulating plans for early detection and for control of mosquitoes. It was time to identify the specific strain to give additional insight into the outbreak and how it might be controlled.

The Experiment Station had virus from four species: two from the mosquitoes captured in Greenwich, one from the crow from Westport, and one from a Cooper's hawk that had been found alive in East Haven by wildlife rehabilitator Karen Hannon but had subsequently died.

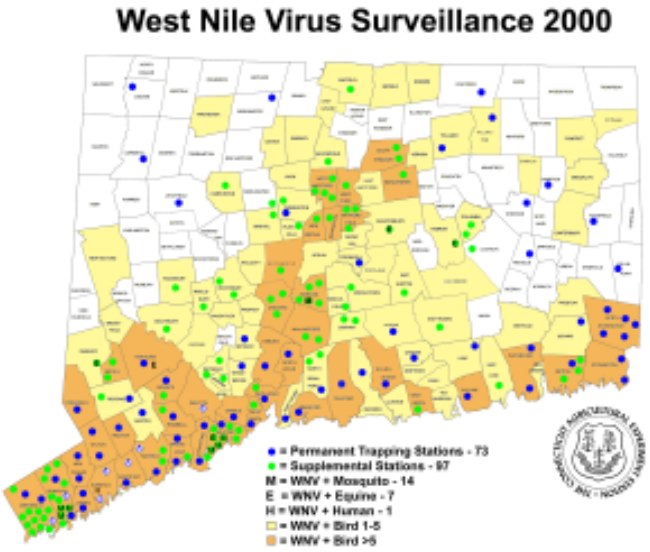
After frost came and the last mosquito was tested, Anderson, Andreadis, Vossbrinck, and several colleagues at Yale and the University of Connecticut wrote a scientific paper documenting the West Nile virus in Connecticut. This paper was published in December 1999 in the same issue of the journal *Science* with another by scientists at the Centers for Communicable Diseases and Prevention detailing the outbreak in New York. The CDC scientists reported the virus matched an unpublished strain recovered from a goose from Israel. These first reports of West Nile virus in the New World will provide the basis of knowledge of the outbreak of 1999 for years to come. The Experiment Station's report in

Science concluded, "Our isolation of WN virus from mosquitoes and birds conclusively documented the presence of this virus in Connecticut during September 1999. If established in North America, WN virus likely will continue to have severe effects on human health and on avian populations, such as American crows and raptors, which heretofore have never been exposed to this virus."

During the winter of 1999-2000, The Experiment Station joined the State Departments of Environmental Protection, Public Health, and Agriculture, and the Pathobiology Department at the University of Connecticut, in outlining a state-wide response plan for surveillance and mosquito control to be followed in 2000.

Mosquitoes have been picked up at 73 surveillance sites throughout Connecticut beginning June 1, 2000 for testing for West Nile virus. In addition, hundreds of birds and bird sera collected by Michael Vasil and Ira Kettle have been tested for the virus. The first West Nile virus of the year from mosquitoes was found in specimens collected in Stamford on July 11, 2000. On July 21, 2000 the finding of the first West Nile virus in a crow from Stamford was announced by the Experiment Station.

The finding of the virus in Stamford, along with reports of the virus in mosquitoes and birds in Massachusetts, New York, and New Jersey documented the fact that the virus had successfully survived the winter in the Northeast.



Map of Connecticut showing where West Nile virus was found during 2000. Data from Experiment Station, Department of Public Health, and Department of Pathobiology at UCONN.

West Nile Virus Facts (September 1999-October 2000)

West Nile virus was found in 28 of 31 crows tested in a 62 mile stretch of Connecticut's coast from Greenwich to Madison and 15 miles inland during 1999. Other bird species testing positive for West Nile virus were a Cooper's hawk from East Haven and a sandhill crane from Bridgeport.

Number of mosquitoes tested during 1999: 45,391. Number of sites where mosquitoes were trapped: 73. The two mosquito species from which WNV was isolated by the Experiment Station during 1999: *Culex pipiens*, the most common suspect vector, and *Aedes vexans*, the floodwater mosquito. *Culex pipiens* prefers stagnant, organic or polluted water such as can be found in catch basins and a variety of backyard habitats.

The towns and the number of trapping sites in each town in Fairfield and New Haven Counties where West Nile virus activity was considered most likely during 1999: An asterisk indicates a bird with WNV was found during 1999. Fairfield County: Greenwich (3*), Stamford (3*), Darien (2*), New Canaan (2*), Norwalk (2*), Wilton (2), Ridgefield (1), Westport (2*), Weston (1*), Redding (1*), Fairfield (2*), Easton (1), Bridgeport (2*), Trumbull (1), Monroe (1), Newtown (1), Stratford (2*), and Shelton (1). New Haven County: Milford (1*), Orange (1*), Woodbridge (1*), Bethany, West Haven, New Haven (1*), Hamden (1*), East Haven (1*), North Haven (1*), Branford (1), North Branford (1), Guilford (1), and Madison (1*).

Over the winter 1999-2000, over 200 mosquitoes, blood from over 1000 songbirds, and serum from over 900 birds were tested for WNV at the Experiment Station, but no virus was found. However, virus was found in New York in mosquitoes by the CDC and the University of Connecticut found, and the Experiment Station confirmed, WNV from a red-tailed hawk that died in February in Westchester County, NY. Virus-infected birds were also reported during early summer 2000 by health authorities in New York and New Jersey.

The first West Nile virus of the year from mosquitoes was found in specimens collected in Stamford on July 11, 2000. At the end of the testing, October 31, 2000, a total of 137,199 mosquitoes had been collected and tested. The virus has been found in four mosquito species: *Culex pipiens, Culex restuans, Culex salinarius*, and *Culiseta melanura*. Towns where the virus was found in mosquitoes and the number of times collected were: Greenwich (1), Stamford (3); Norwalk (2), Fairfield (1), Milford (3), Shelton (1), Meriden (1), and Westport (1). In addition West Nile virus affected horses in Bethel/Danbury, Cheshire, Glastonbury, Hebron, Middlefield, Milford, and Norwalk. A total of 1,105 birds tested positive for West Nile virus in 109 communities in all eight counties.

The Bird and Butterfly Garden at Lockwood Farm surrounds visitors with texture, shape, color, and scent. Garden benches, nestled in patches of dappled sunlight allow respite from the world and allow contemplation of its winged and floral beauty.

Bird and Butterfly Garden at Lockwood Farm demonstrates plantings to attract wildlife

By Carol R. Lemmon

Amidst the neat rows of research plots at The Connecticut Agricultural Experiment Station's 75-acre Lockwood Farm is a one-half acre wildlife oasis: a Bird and Butterfly Garden where more than 34 species of butterflies have been seen fluttering among the glorious array of color and sipping nectar from the myriad of blossoms that dole out sustenance.

Dragonflies glint like jewels with their aerial displays over the open pools where they deposit eggs that will soon develop into dragon-like nymphs and will clean the pools of mosquito larvae.

A hummingbird moth, aptly named, looking like a miniature hummingbird but with antennae, hovers before the blossoms for its share of nectar.

Bluebirds bathe at the shallow seep, and a song sparrow offers a song, phonetically akin to,"maids, maids, maids, put on your tea kettle, kettle, kettle...." from a nearby white pine.

Turkeys, deer, and raccoons leave footprints in the sand around the swampy seep as evidence of a nocturnal or early dawn visit where they stooped to drink at the only water source in the area.

Fat-bellied tadpoles in the upper pond produce a pair of hind legs, an indicator their aquatic life stage will soon be over.

Last year a muskrat took up temporary residence under the footbridge crossing one of the connecting streams while considering whether this small refuge might become home.

View above the lower pool during the summer.

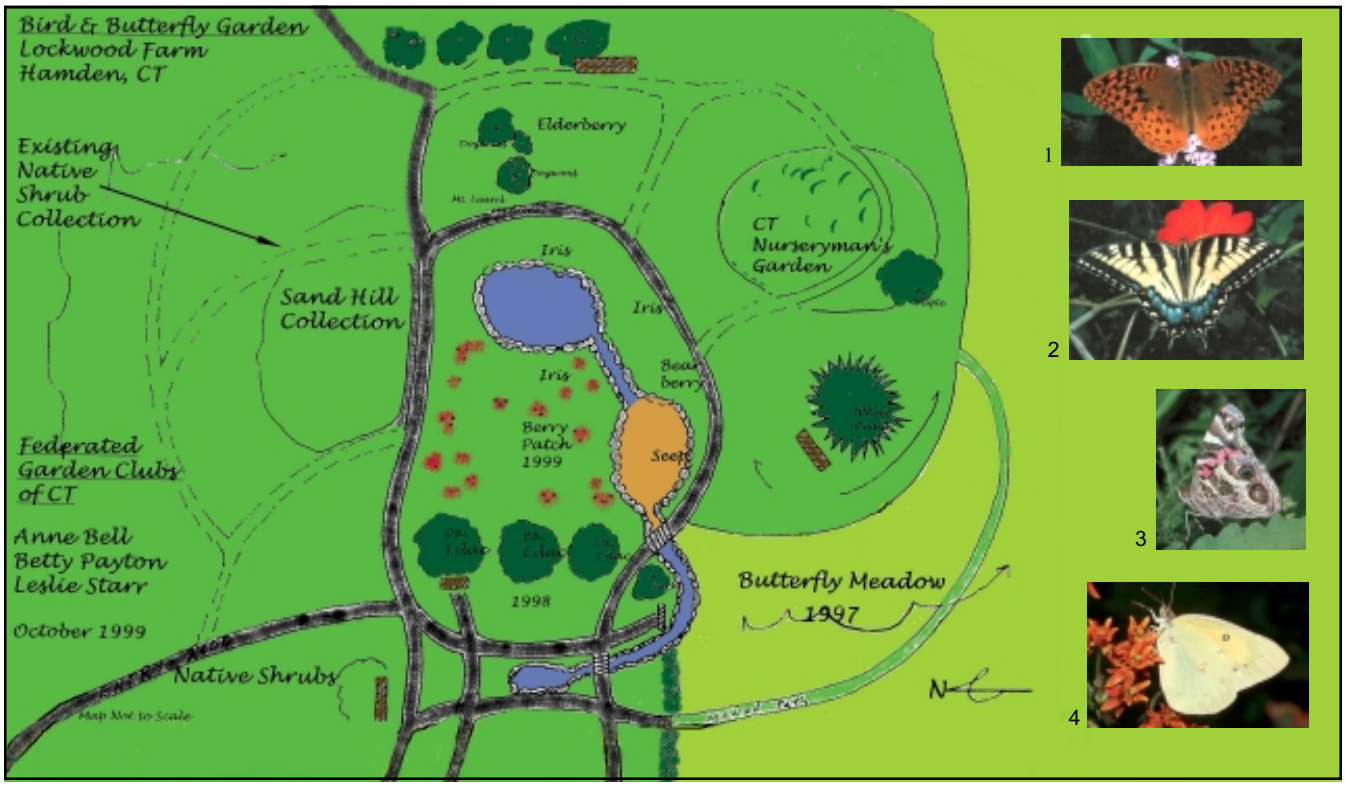
The concept of a Butterfly and Bird Garden at Lockwood Farm was envisioned at Plant Science Day 1996

With all of this flourishing wildlife, this small sanctuary may appear as if it has always been there. But actually, the concept of a Butterfly and Bird Garden at Lockwood Farm was envisioned at Plant Science Day 1996 by Jack Faulkner of Darien. A long-time birder and butterfly enthusiast, he proposed the idea to the Station Director, Dr. John F. Anderson, who wholeheartedly agreed. Faulkner's wife Susan, past president of The Federated Garden Clubs of Connecticut, suggested that garden-club women might help. Plans were made that winter to incorporate the existing Native Shrub Collection and the Connecticut Nursery and Landscape Association Garden with the new Bird and Butterfly Garden and meadow plantings.

The overall water feature was designed by landscape architect Betty Payton of the Evergreen Garden Club. She incorporated a swamp-filtered seep between the upper and lower pools connected by a small stream. Farm Manager Edward Naughton and his staff did the construction. The water from the upper pool runs down the stream to the lower pool and is pumped back via buried pipes. Betty wanted to try a swamp-filtered seep to keep the water clean, like a natural wetland, using bacteria and emergent plants to act as a dynamic living filter. A seep area was created below the upper pool. This shallow site was created by digging a pool, lining it with black plastic, and then filling it with coarse sand. The sand was adjusted to allow the water to flow over it, at a depth of just a couple of inches, through the center of the pool.

Wetland plants, such as forget-me-not (*Mysotis palustris*), blue flag, (*Iris versicolor*) and cardinal flower (*Lobelia cardinalis*), were buried along the edges in the sand using only the potting soil that came with them. Several wetland sedges were planted in the center of the pond and the stream of water passes through these fine grass-like blades. Flat rocks were placed in the seep, just above water level, to give birds a place to drink or to preen after bathing.

The sand along the edges of this seep remains moist and gives honey bees and butterflies a safe place to alight. They drink by inserting probosci into the moist sand and suck up



The plan for the entire Bird and Butterfly Garden at Lockwood Farm as it will appear when finished. The darker paths have been completed. At the right are four butterflies that can be seen in the garden. 1. Great spangled fritillary; 2. Eastern tiger swallowtail; 3. American lady; and 4. Orange sulphur. Garden diagram by Anne Bell. Butterfly photos by Jeffrey Fengler.

water and dissolved minerals. This seep is the water source used by most of the wildlife. Animals can walk safely into the seep on the packed sand, and the water is shallow enough for birds to bathe. The seep and the pools are the only water sources on the farm. Clean water leaves this shallow seep by riffling downhill to the lower pool.

The pools are not deep enough to keep over-wintering fish. They are more like vernal pools harboring tadpoles, dragonfly and damselfly larvae. Lily pads give shade and shelter to its denizens. A thin layer of algae that often grows on the bottom of these pools provides food for the invertebrates and hides the unsightly black plastic liner. The pools, seep and connecting streams are outlined in rocks from the farm to hide the plastic edges, and to act as a safety feature so that no one will accidentally step in them.

Anne Bell, a member of The Garden Club of New Haven, designed the plantings for the Butterfly Garden and a berry patch for birds. She included an interconnecting system of 740 feet of pathways that meander throughout the gardens, meadow, existing native shrub plantings, and The Nursery and Landscape Association Garden. Many of these paths are wheelchair accessible; the others will be made accessible in the future. With her skills as a landscape designer and her knowledge of native and cultivated plants, Anne pulled all of these ingredients together to produce magic: the garden is enchanting.

More than sixty annuals, perennials and woody plants make up the Butterfly and Bird Garden. Seven 'Burgundy' butterfly bushes (*Buddleia davidii*), which are long flowering shrubs, were used as a foundation of color and nectar. The perennials butterfly weed (*Asclepias tuberosa*) and 'May Night' salvia (*Salvia nemerosa*) also are nectar sources and have deep rich colors which are attractive to butterflies. Butterfly weed has the added advantage of being larval food for the monarch butterfly. The cardioglycosides found in this milkweed plant are sequestered by the larvae and passed on to the adult monarch, giving protection from bird predation. Young birds that eat monarchs ingest these milkweed chemicals and become violently ill. Subsequently, the birds avoid orange butterflies. The 'May Night' salvia produces nectar in deep blue blooms from late May until frost. The most important annual larval food source in the garden is parsley. Black swallowtail larvae sit on parsley plants camouflaged as bird droppings. As the larvae molt and become larger they will be susceptible to bird predation as they become shades of green, black and yellow and will then feed amidst the foliage to avoid discovery. Another annual, 'Cut and Come Again' zinnia, is one of the strongest flower/nectar producing plants in the garden. Being partial to perennials, I was surprised to see how important beds of zinnias are in the garden. They bloom in late May and still flourish in late fall, providing nectar for late migrating butterflies, such as monarchs. One autumn the zinnias were still glorious on November 17. I can assure all that I now grow these annuals in my garden at home.

Leslie Starr of the Haddam Garden Club planned the butterfly meadow, converting an idle farm plot to wildlife habitat with larval and nectar food plants. On this site, garden club volunteers and Station and farm staff planted plugs of purple cone-flower, (*Echinacea purpurea*), tickseed

The bog seep, frequented by birds and animals, at the left. Above, the butterfly meadow can be seen in the background looking up from the lower pool.

The bird garden around the upper pool includes the previously established native shrub garden, as well as a new collection of native shrubs that provides seeds and berries.

(*Coreopsis lanceolata*), bee balm, (*Monarda didyma*), and butterfly weed (*Asclepias tuberosa*). The meadow changes its look frequently. Daisies and fleabanes often dominate. It is amazing how a meadow will bloom with wildflowers, grasses and sedges that were not planted there. Every year several new plants arrive, seemingly by magic. Actually, the seeds find their way to the meadow by wind, in bird droppings, snagged on an animal's fur, or perhaps carried on a sneaker lace or pant cuff.

The grasses are very important. A butterfly meadow should be about two thirds grasses and one third wildflowers. Nearly all skipper butterflies feed on grasses as their only larval food source. They overwinter in the base of the grasses and become adults the following spring.

The meadow is mowed once every three years in the early spring before the wildflowers emerge to prevent woody growth. Allowing the seed heads to remain on the wildflowers and grasses at the end of the season gives the overwintering birds a food source. All invasive species, however, are removed immediately before they gain a foothold; they are called invasives, not just aliens or non-natives, for a reason.

The bird garden around the upper pool includes the previously established native shrub garden, as well as a new collection of native shrubs that provides seeds and berries. We planted blueberries, huckleberries, Virginia sweetspire, crab apples, beach plum, and bearberries to attract migrating fall birds. Bee balm (*Monarda didyma*) has been added as a nectar source for hummingbirds.

The farm doesn't have the habitat for many nesting birds, but some chipping, house and song sparrows, bobolinks, kestrels, robins, mockingbirds, bluebirds, American and fish crows, morning doves, and goldfinches have nested on the farm and have visited the garden. Flocks of cedar waxwings and red-tailed hawks come through in fall migration.

The bird garden shrubbery was chosen to attract possible butterfly-eating birds to the garden only in late autumn when butterflies are less likely to be there.

On Plant Science Day, August 4, 1999, 19 species of butterflies were evident. Nine eastern tiger swallowtail adults were seen at one time, either nectaring on the buddleia or zinnias. Skippers, small, dark, scarcely known butterflies, were flitting everywhere.

Some of the 34 different species of butterflies arrive early in the spring, such as Juvenal's duskywings and the common sootywing. The hobomok skipper shows up about mid-May through June. There have been several early arrivals to the garden of a normally uncommon butterfly called the variegated fritillary. These sightings occurred in May. Normally, only great spangled fritillaries appear from mid-June until mid-September. These large rusty-orange fritillary butterflies lay their eggs on violets. Their caterpillars feed at night on violets, their only larval food source. Violets are abundant at the garden for this reason. The most common butterflies are the eastern tiger swallowtail, which the Federated Garden Clubs of Connecticut has proposed to be our state butterfly, and the large black and iridescent blue spicebush swallowtail. About mid June the monarch becomes abundant, having several broods about a month apart until the last brood

migrates to Mexico in September or early October. American lady, viceroy, pearl crescent, gray hairstreaks, and American coppers are also commonly seen there. There are also many abundant skipper butterflies with wonderfully mysterious names such as long dash, northern broken dash, fiery, zabulon, crossline, dun, and Pecks.

The look of the garden changes monthly as different plants come into bloom and temporarily dominate. Some bloom throughout the summer but become majestic with berries and seedpods in the early fall.

Visitors are encouraged to explore the farm and especially visit the butterfly and bird oasis. They may catch a glimpse of the two cottontail rabbits, which can be seen in the greenery in front of the garden. They often slip out into the grassy roadway to nibble on the clover. The resident chipmunk may scurry by as if on a mission. He often sits on the footbridge to feed, leaving messy seed shells behind. The brown thrasher can often be seen in the native shrub garden taking a daily dust bath. With close observation, this little dustbowl can be found. In the fall, when twilight comes early, the red fox which visits the seep every day may been seen along with the resident deer that thinks the lily pads in the pools and the lovely blue forget-me-nots are the salad course for its dinner.

The garden is open to the public during the station hours, Monday through Friday from 8:30 a.m. to 4:30 p.m.

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