Biopesticides Supporting Trees and the Arborist Industry

2018 Garden State Shade Tree Conference
International Society of Arboculture

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Center for Integrated Pest Management

Presentation Overview

- Biopesticides Introduction
- American Chestnut – Chestnut blight
- American Elm – Dutch elm disease
- Ash Trees - Emerald ash borer
- Gypsy moth
- Pheromones
- Codling moth
- Root Rot

Today I will be discussing the comeback of the American chestnut tree and the tools that are bringing this tree back from near extinction,
Similarly, we will discuss the plight of the American elm and the biopesticide and crossbreeding tools currently in use to develop more pest resistant elms.
I'll also discuss ash trees, troubled by the emerald ash borer, and how biopesticides are leading the way to saving specimen trees.
There are many biologically-based pesticides used to control gypsy moth populations, including pheromones.
Speaking of pheromones, we will briefly discuss how they can be used to trick and trap several pests.
We will look at codling moths and tools for their control.
And lastly, a fungus to prevent root rot in managed forests.

Biopesticides: Eco-Friendly Pest Control

- Biopesticides include naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants)
- **Biochemical pesticides** are naturally occurring, non-toxic substances that include those that interfere with mating (insect sex pheromones) and plant extracts that attract insect pests to traps.
- **Microbial pesticides** contain a microorganism (e.g., a bacterium, fungus, virus or protozoan) that controls pests.
- **Plant-Incorporated-Protectants** are pesticidal substances that plants produce from genetic material that has been added to the plant.

See www.epa.gov/pesticides/biopesticides

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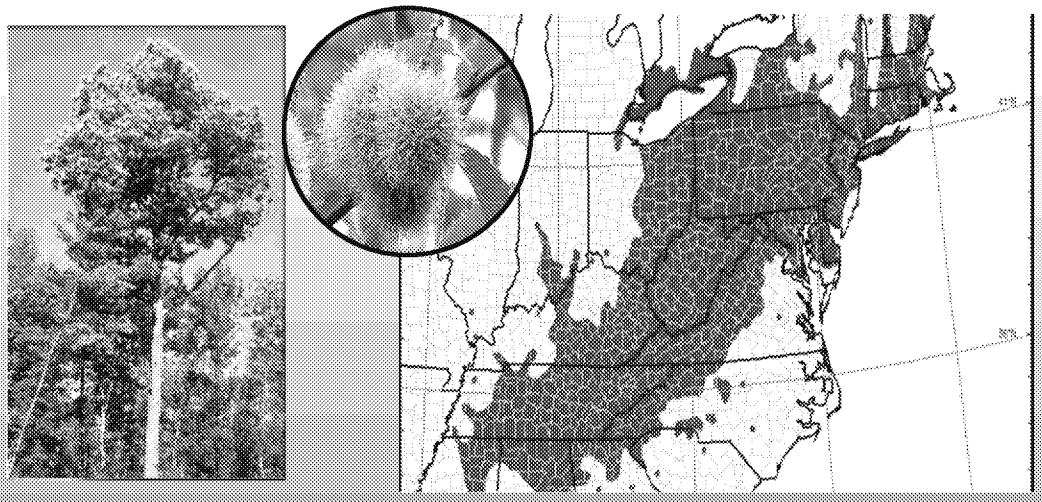
Biopesticides are often important components of integrated pest management (IPM) programs, and have received much attention as substitutes to synthetic chemical pesticides.

American Chestnut: Research and Restoration

American chestnut once covered an extensive range across the US and was a valuable source of wood, nuts for human and wildlife consumption, and driver of local economies.



(*Castanea dentata*)

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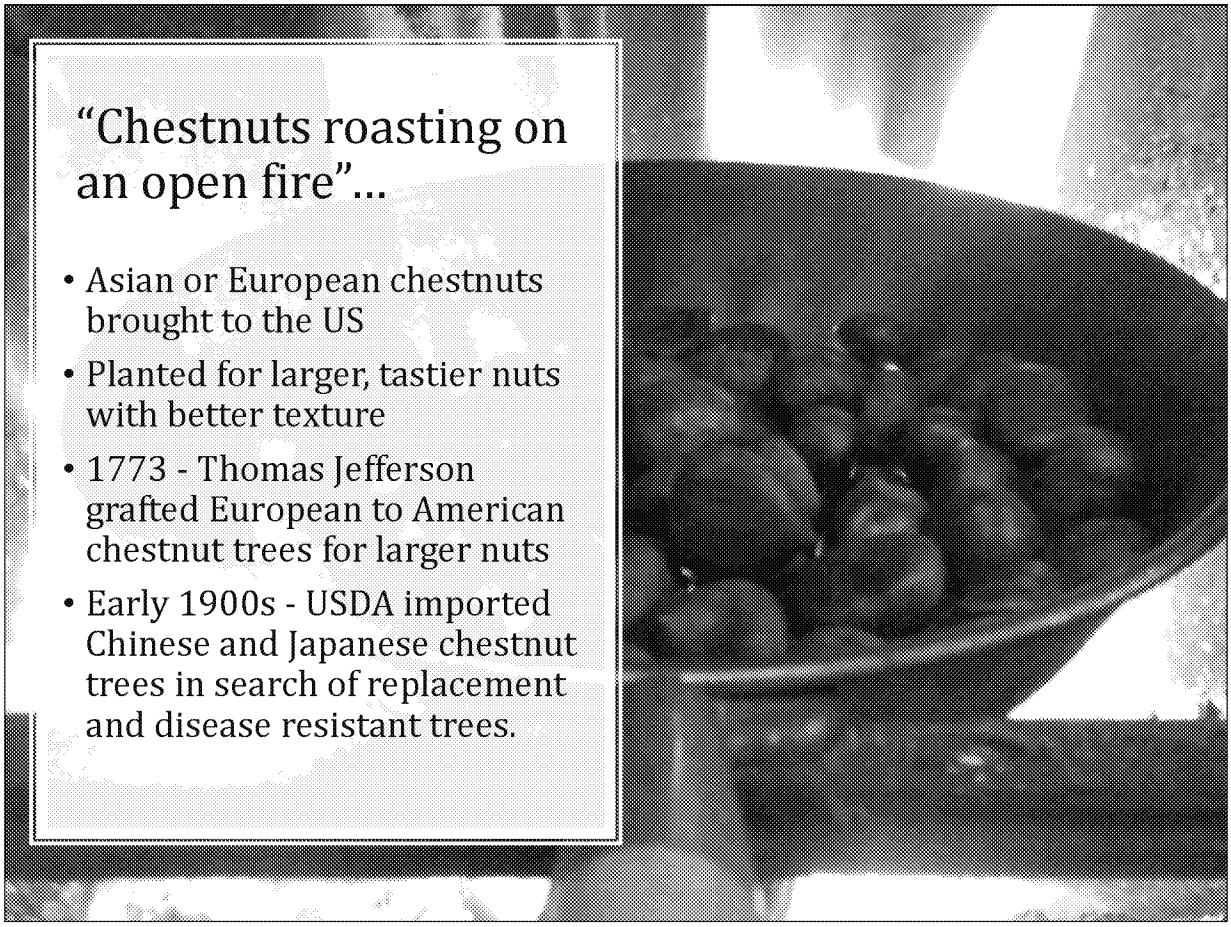
Let's begin our forest journey with the American chestnut.
Before the early 1900s, the American chestnut was among the most majestic hardwood tree in woodlands from Maine to Georgia.
4 billion trees across more than 30 million acres. They were known as "the Redwoods of the East".
Grew to over 115 feet in height, diameters exceeding 12 feet, attained an age of 200-300 years.
They were prized for their pest (termite and carpenter ant) resistant timber.
July, they would display panicles of large, white flowers bursting with fragrance.
Now the remnants of the once proud American chestnut are little more than an early succession-stage shrub



Although Native Americans, early settlers and wildlife found the annual nut crop as a nutritious form of sustenance – they are a bit "strong" in flavor. Definitely an acquired taste.
So What about the popular "Chestnuts roasting on an open fire?"
They are typically from Asian or European chestnuts brought to the US and planted specifically for the larger and tastier nuts.
Back in 1773, Thomas Jefferson grafted European chestnuts branches to American chestnut trees for larger nut size and texture.
In the 19teens, the USDA imported both Chinese and Japanese chestnut trees in search of replacement and disease resistant trees .
Beginning of the breeding program by USDA and chestnut breeders to replace dead and dying American chestnuts.

American Chestnut: Blight

- Chestnut blight accidentally introduced into the U.S with the importation of Asian (Japanese) chestnut logs for lumber.
  - Discovered in 1904 in the Bronx Zoo
  - Widespread by 1905
- Breeding program begun by USDA and chestnut breeders
- USDA and Connecticut Experiment Station researchers increasing resistance through crosses and backcrosses

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The chestnut blight caused by the exotic fungal pathogen, Cryphonectria parasitica was accidentally introduced into the United States in the early 1900s with the importation of Asian (Japanese) chestnut logs for lumber.
Initial discovery of the blight was in 1904 in the Bronx Zoo and by 1905 the disease was widespread.
This also was the beginning of the breeding program by USDA and chestnut breeders, whose work was eventually expanded by the Connecticut Agricultural Experiment Station.
USDA, Connecticut researchers, and other breeders began to increase resistance through a succession of crosses and backcrosses
The American Chestnut Foundation is working through genetic backcrosses to improve the habit and phenotype / diversity of the final tree type for planting.

Signs of chestnut blight
(Image: Joseph O'Brien, USDA Forest Service, Bugwod.org)

American Chestnut:
Research

- 1970s - biopesticide found in France
- A virus was found that inhibits the growth of the infecting chestnut blight fungus - called hypovirulence
- Experimental virus causes blight cankers to stop expanding
- Tree's natural defenses wall off the disease protecting the cambium
- Tree heals over the blight cankers with lumpy bark tissue
- Once hypovirulence has been established in chestnut orchards with blight population, the spores can be easily carried by insects, or anything that walks, flies, or crawls

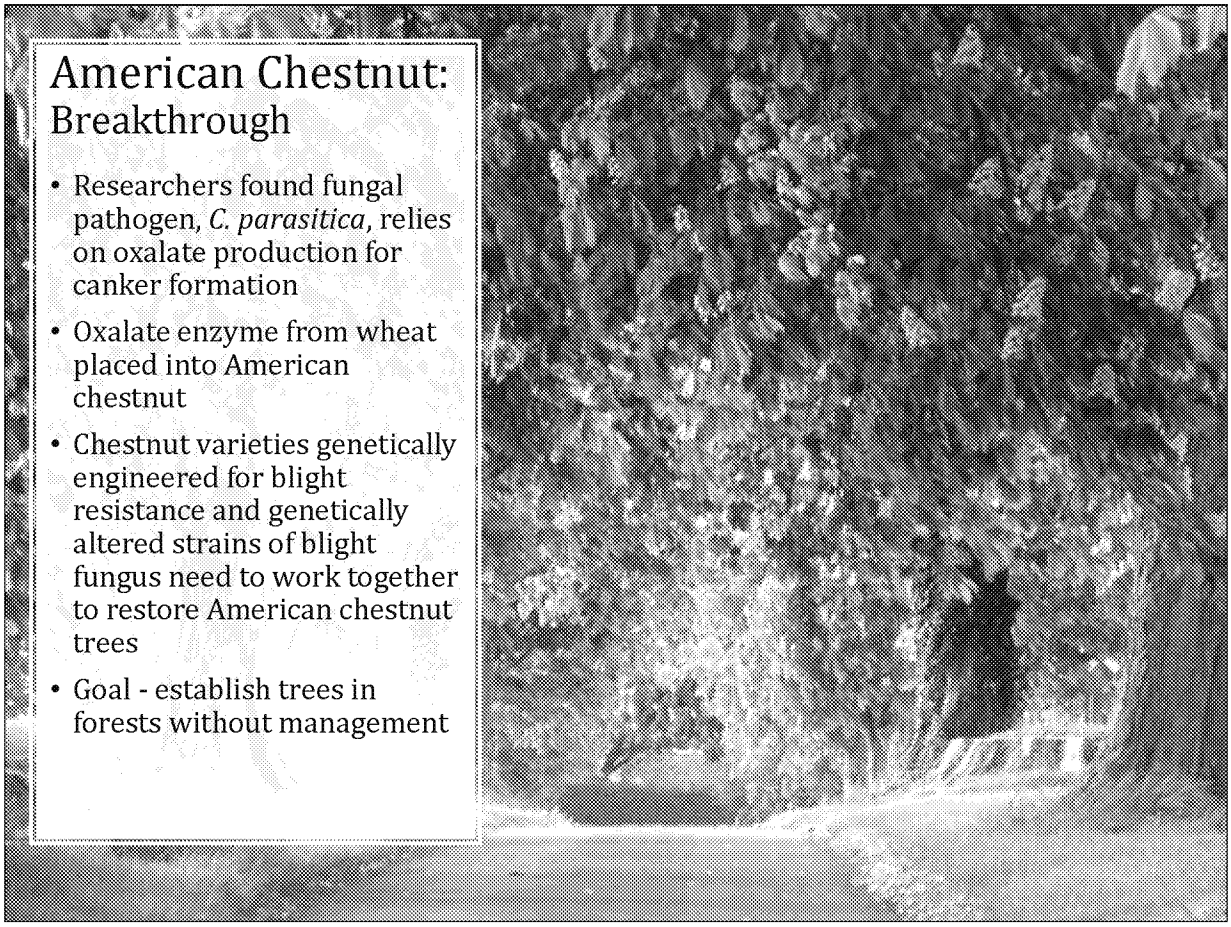
In the 1970's, a biopesticide was found in France. Discovery of a hypovirulent virus that inhibits the growth of the infecting Chestnut blight fungus. First used in Europe – then sent to the US for Experimental use. (Under USDA permit)
The cankers stop expanding, and the tree's natural defenses wall off the disease, successfully protecting the tree's delicate cambium layer.
Healing over the blight cankers with lumpy bark tissue.
Once hypovirulence has been established in chestnut orchards with a blight population, the spores can be easily carried by insects, or anything that walks, flies, or crawls .
This virus was very effective in Europe on European elms, but unreliable in the US on American elms.
Although the virus was not an EPA-registered biopesticide, it is an example of how biological agents can be discovered and the scientific breakthroughs they can initiate.
Stay with me as I explain.

American Chestnut:
Cross-Breeding

Connecticut Experiment Station breeding program:

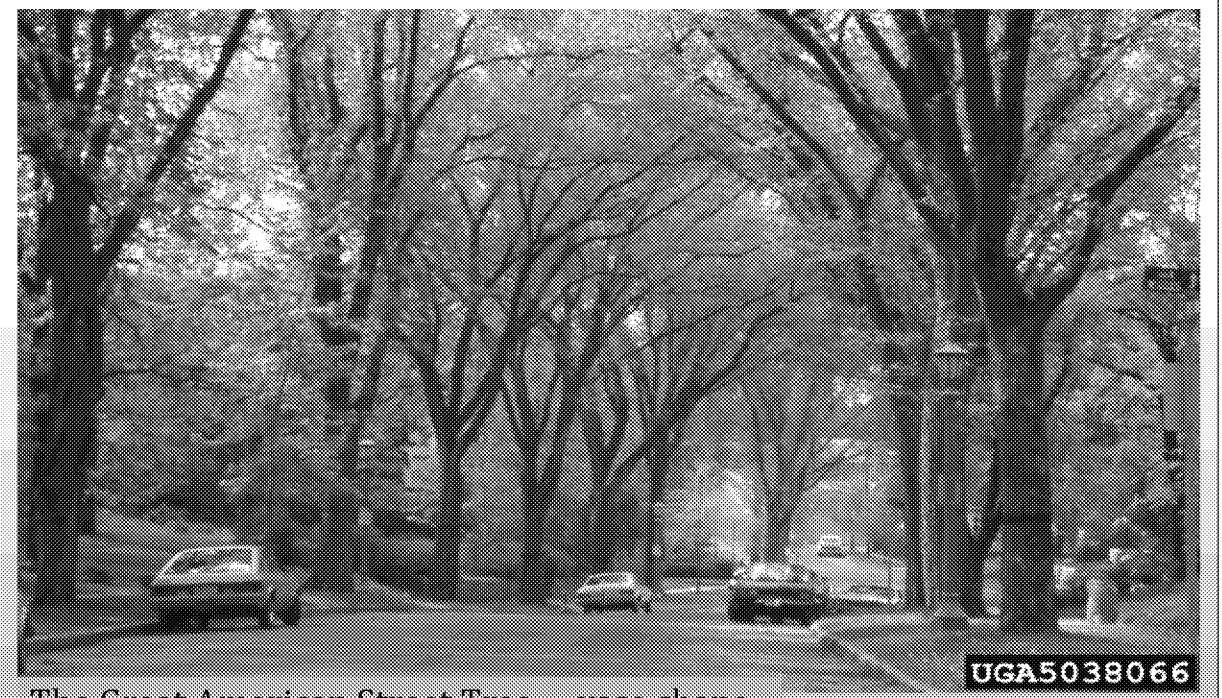
- Inoculated 5-year old trees with blight
- Even if new blight cankers formed, protective callouses developed and many trees survived
- Document which trees survived
- Resistant trees used for back-cross breeding program
- Found dsRNA strains were causing cankers

As part of the Connecticut Experiment Station breeding program, researchers inoculated 5-year old trees in the field with the blight.
They documented which trees survived.
Even if new blight cankers formed, they found that callouses subsequently formed, and many of the trees survived.
The trees showing increased blight resistance were used in the continuing back-cross breeding program.
A further breakthrough occurred when dsRNA viruses in the C. parasitica strains were found to cause cankers, but not kill the trees.



By the use of the hypovirulent virus that I mentioned before, Connecticut Experiment Station researchers found that the fungal pathogen, Cryphonectria parasitica, relied on oxalate production for canker formation and invasion of tissues.
The State University of New York was able to move the science forward by isolating oxalate oxidase from wheat germ and placing the genes into the American chestnut. Creating a transgenic American chestnut.
The chestnut varieties genetically engineered for blight resistance and genetically altered strains of the blight fungus need to work together to help to restore the long-treasured American chestnut tree.
The transgenic trees express the oxalate oxidase enzyme that neutralizes the oxalic acid that is released by the fungus. It's this acid that, without neutralization, would damage and eventually kill the tree.
The oxalate oxidase enzyme neutralizes the acid after the fungus has released it, so the acid does only minimal damage to the tree tissues.
The transgenic chestnut is changed so that it can tolerate the fungus that is continually present. It is basically "blight tolerant"
The goal is to keep the genetic diversity in the surviving American chestnut population by crossing the blight tolerant (resistant) trees to the surviving trees – thus preserving wild American chestnut genes.
This is the best hope, in over a century, for restoring the species.
After over century of absence, American chestnuts are now taking root across America.
Each of the seven new hybrid chestnuts varies with regard to blight-resistance. The transgenic "Restoration" chestnut, is a sixth generation hybrid.
The State University of New York and the American Chestnut Foundation are seeking assistance and advice from EPA, FDA and USDA on moving this technology forward under the Coordinated Framework for the Regulation of Biotechnology.

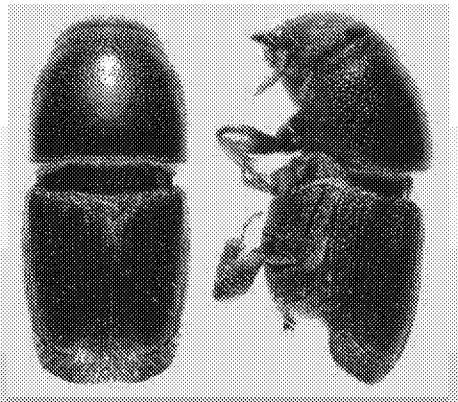
American Elm: Overview



The Great American Street Tree – vase shape, over 100', quick to establish.

Early American settlers so valued elm trees that, by the 1700's, they had begun planting them in their front yards.
Elms are the perfect street tree. They grow to 100+ feet in a vase shape that enables them to stay out of the way of delivery trucks.
Grow quickly, reaching close to 50' in 15 years.

American Elm: Bark Beetle | Dutch Elm Disease



Elm bark beetle adult
(Photo courtesy Guy Hanley, Minot State Univ.)

- 1919 - European elm bark beetle crossed the Atlantic in logs from the Netherlands
- Elm survivors in Boston, New York City, Chicago, Washington, D.C., and Philadelphia – due to repeat fungicide treatments
- Some trees show genetic resistance to Dutch elm disease – basis for crossbreeding

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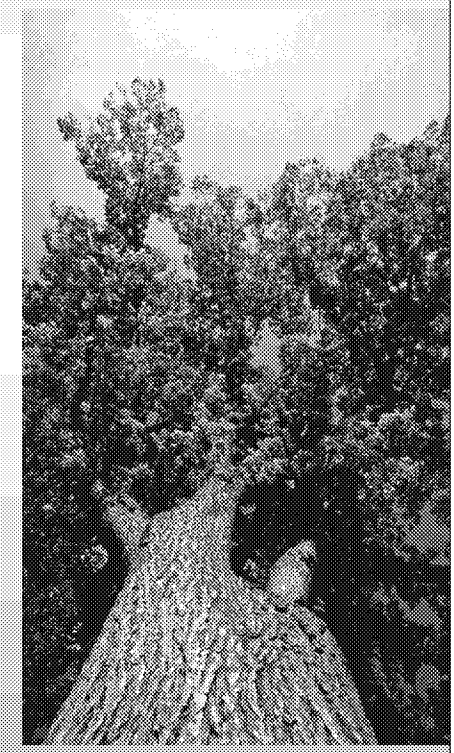
Millions of elm trees were lost to Dutch elm disease carried by invasive European elm bark beetle.
There are still a few elms remaining in New York City's Central Park, the National Mall in Washington D.C, and Longwood Gardens, PA.
Their survival is due to repeated, fungicide treatments to ward off Dutch elm disease.
Other survivors - the largest natural elm forest, with about 200,000 trees, survives outside of Winnipeg, Canada.
Some juvenile elms continue to struggle for life along river banks and in yards, sprouting from roots of older trees.
Scattered survivors show some genetic resistance to the disease – basis for cross – breeding and restoration.

American Elm:
Saving Specimen Trees

- 2005 - EPA registered the fungus, *Verticillium* Isolate WCS850, to protect trees against Dutch elm disease
- Injected into healthy elm trees to stimulate the tree's natural defenses against the disease
- Application via a closed system gouge gun
- Must be applied annually
- Does not harm the treated tree, humans or the environment
- Good for saving a specimen trees



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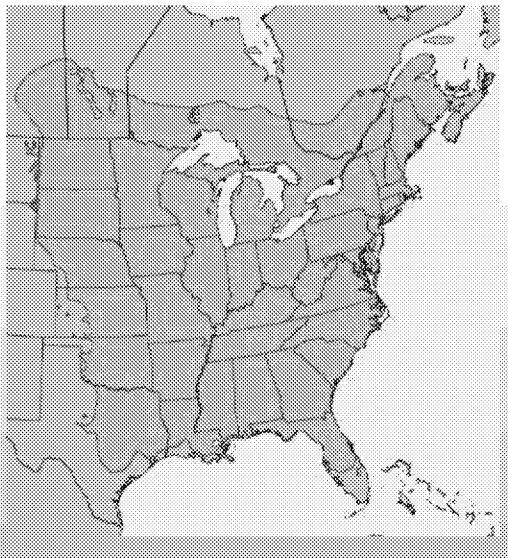
In November of 2005, the EPA registered the fungus, Verticillium Isolate WCS850, to protect trees against Dutch elm disease.
When injected into healthy elm tree trunks, it stimulates the tree's natural defenses so that the tree becomes resistant to the organism that causes the disease.
Application is via a closed system gouge gun which injects a few drops of the fungal suspension into the tree's vascular system.
Applications must be made annually.
The biopesticide does not harm the treated tree, humans, or the environment.
While it is good for saving specimen trees, cost and the practicality of application make it not feasible for use in entire forests.

American Elm: Cross-Breeding

- US Forest Service has been conducting restoration experiments for half a century
- Take cuttings from Dutch elm disease survivors
- Cross with existing disease-resistant trees
- Propagate saplings to test for disease resistance
- Nature Conservancy's Connecticut River Program plants, nurtures and monitors juvenile trees



It is so important to restore these trees, that the US Forest Service has been conducting restoration experiments for half a century.
A long and intensive program - and occurs primarily in the USDA Forest Service lab in Delaware, Ohio.
The USDA scientists take cuttings from Dutch elm disease survivors for propagagion.
It takes years for the surviving little saplings to grow.
Then the scientists make crosses with other Dutch elm disease resistant trees.
After propagating thousands of elm saplings, they test them for resistance to the disease.
Nature Conservancy's Connecticut River Program plants, nurtures and monitors the juvenile trees.
Each tree is GPS located to enable monitoring.

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American Elm: The Future Begins Now

- Forest Service is half-way to goal of 15 Dutch elm disease-resistant varieties
- 7 commercially available varieties
  - 'Princeton' and 'Valley Forge' were the first
  - Newer varieties include New Harmony, Prairie Expedition, and Saint Croix
- More varieties are needed for long-term survival, so trees will evolve resistance, as the pathogen evolves.

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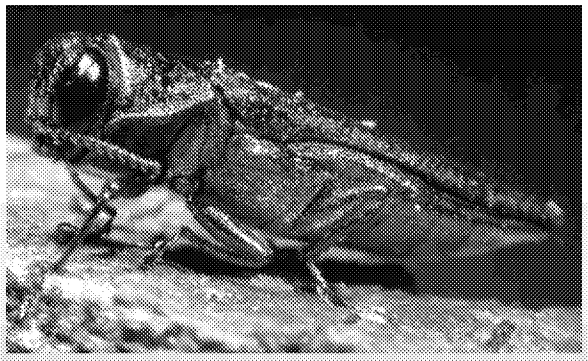
The Forest Service is half-way to its goal of 15 Dutch elm disease resistant varieties.
They have 7 varieties commercially available.
The first resistant varieties successfully bred were 'Princeton' and 'Valley Forge'
Other new varieties include New Harmony, Prairie Expedition, and Saint Croix
More varieties are needed for long-term survival in the wild, so through natural selection, the trees will evolve resistance, as the Dutch elm disease pathogen evolves.

Ash Trees: Emerald Ash Borer

- Emerald ash borer is a wood-boring pest native to Asia
- Found in Michigan in 2002
- Swept through the midwest, mid-Atlantic, and northeastern US and devastated almost every ash tree in its path.
- 20+ million dead trees.



- By the time trees show signs of decline, it is too late.
- 95% of ash trees hit by the borer die within 5 years.
- The only way to save your ash trees is to be proactive.

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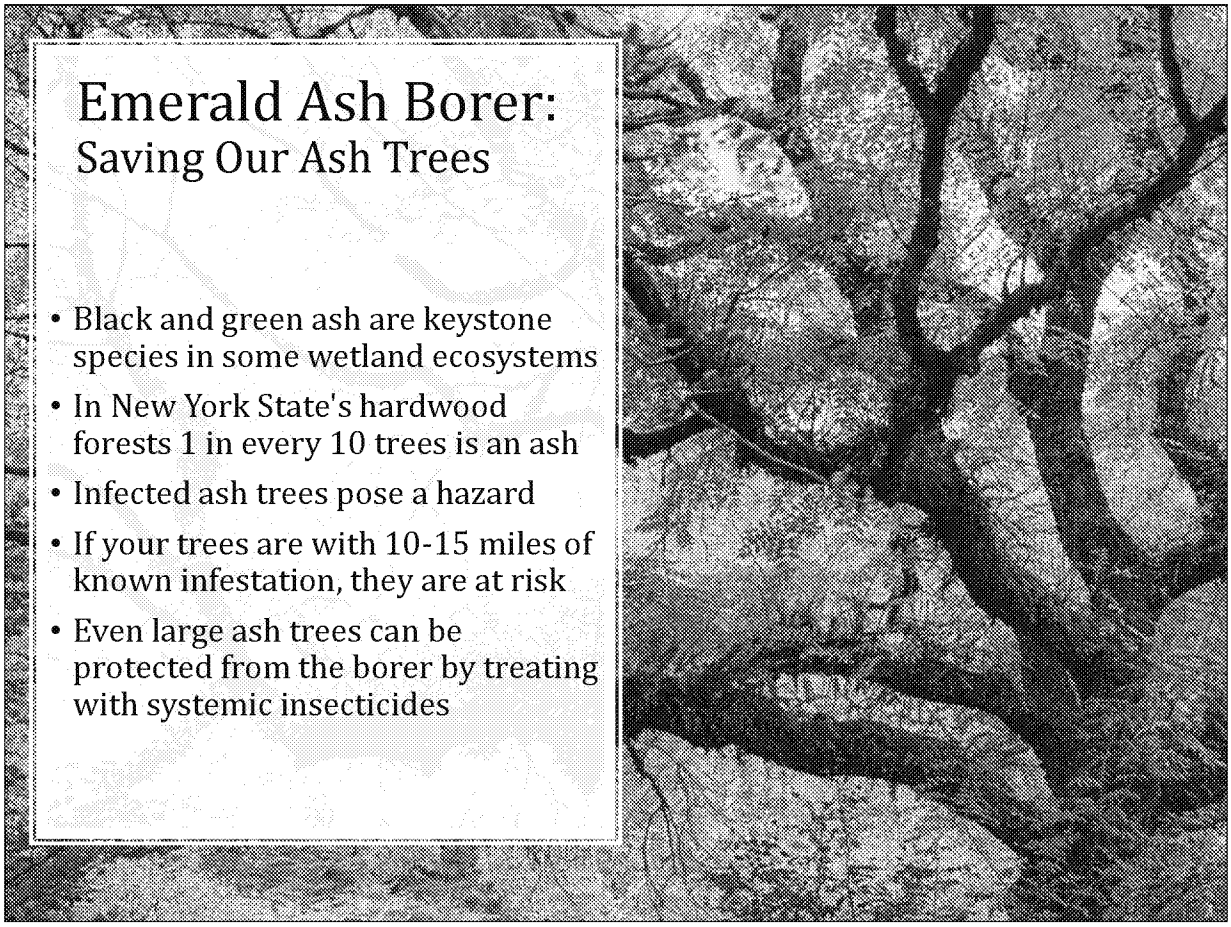
Emerald ash borer, Agrilus planipennis, a sporadic wood-boring pest native to northeastern Asia, was found attacking ash trees (Fraxinus spp.) in southeastern Michigan in 2002.
The borer swept through the midwest, Mid-Atlantic, and northeast devastating nearly every ash tree in its path.
20+ million trees dead.
By the time trees show signs of decline, it is too late
Because, as the borer population grows and increases within a tree, their galleries interfere with the tree's transport of nutrients and water. Tree canopies become thin because fewer leaves can be sustained by the tree.
95% of ash trees hit with Emerald ash borer will be dead in 5 years
The only way to save your favorite ash trees is to be proactive.



Black, green, white and blue ash (Fraxnus nigra, F. pennsylvanicum, F. americana, F. quadreagulata) are keystone species in some ecosystems, so their loss could mean the loss of significant ecosystems.
For example - In New York State's hardwood forests 1 in every 10 trees is an ash,
Infected ash trees are hazardous.
If your trees are with 10-15 miles of known infestation, your trees are at risk.
Even large ash trees can be protected from the borer by treating with systemic insecticides.

Emerald Ash Borer:
Systemic Insecticides

- Large ash trees can be protected with systemic insecticides
- EPA registered pesticides for this use are:
  - Imidacloprid - soil treatment
  - Dinterfuran - bark spray/ soil treatment
  - Emamectin nenzoate - tree injection
- Applied annually (soil/sprays) or biannually (injections)
- The insecticide must be translocated within the tree to allow larvae to eat

(Image: Howard Russell, Michigan State Univ., Bugwood.org)

Three primary systemic pesticides for controlling the emerald ash borer are:
1.) Imidacloprid - a soil treatment
2.) Dinterfuran - a bark spray and soil treatment
3.) Emamectin Benzoate - a tree injection.
Trees must be treated annually or, in the case of emamectin benzoate trunk injections, every other year.
To be effective, the insecticides must be transported within the tree.
The insecticides MUST be eaten by the larvae residing in the insect galleries where they devour the phloem and xylem tissues that the trees require to transport their nutrients and water.
As the borer population grows and increases within a tree, their galleries interfere with this transport system up into the canopy and also inhibits conveying insecticides.

Emerald Ash Borer:
Biochemical Pesticide

- Biochemical pesticide made with azadirachtin extracted from neem tree seeds
- Controls the borer via injection into conductive tissue - lasts for two years.
- Diffusion through tree via sap movement
- Limits the spread, development and reproduction
- EPA Restricted Use Product

Emerald ash borer galleries
(Image: Eric R. Day, Virginia Polytechnic Institute and State University, Bugwood.org)

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There is one biopesticide, made with azadirachtin molecules extracted from neem tree seeds, that can be used to control the emerald ash borer.
An azadirachtin treatment via injection into the tree's conductive tissue can be effective for two years.
It diffuses throughout the tree by sap movement.
This biopesticide limits the spread, development and reproduction of the borer.
It is a Restricted Use product.

Emerald Ash Borer:
Biochemical Pesticide Efficacy

Year 1

- 95% of larvae die
- Females lay fewer eggs
- 99% eggs are not viable

Year 2

- Any surviving larvae do not complete development
- Number and length of larval galleries significantly lower

Long-Term

- Regular treatment may be necessary for 6-10 years

Azadirachtin also EPA registered to control:

- gypsy moth
- tent caterpillars
- spruce budworm
- arborvitae leafminer
- jack pine budworm
- sawflies (birch leafminer)
- European elm scale
- hemlock wooly adelgid
- elm leaf beetle

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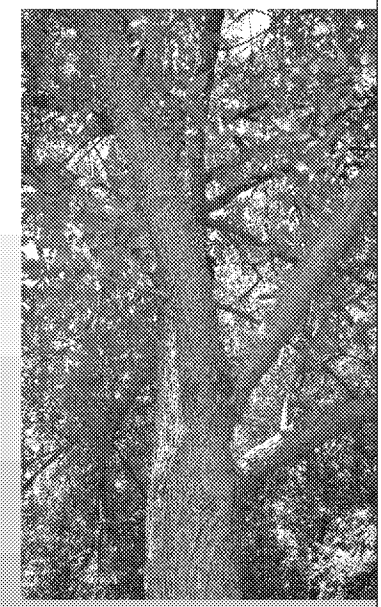
Azadirachtin has been shown to be highly effective against the borer.

In first year:
95% of emerald ash borer larvae feeding on treated tissue do not complete development and die
Adult females lay significant fewer eggs
99% eggs are not viable

In the second year: EAB larvae do not complete development
Number and length of galleries are significantly lower
Regular treatment may be necessary for 6-10 years
It can be used as part of an IPM program that emphasizes the monitoring of pest numbers.

Emerald Ash Borer:
Microbial Pesticide

- Entomopathogenic fungi - the major mortality factor of emerald ash borer in wild Michigan populations
- Adults susceptible to *Beauveria bassiana* and *Metarhizium anisopliae*
- Isolate *B. bassiana* GHA kills adults
- Sub-lethal effects:
  - longevity reduced from 22 to 13 days (females) and 28 to 14 days (males)
  - fewer eggs produced
  - Longer larval development time
- Reduces borer numbers and slows ash decline through trunk and foliar applications
- Useful for healthy trees and in slowing progress in core zones



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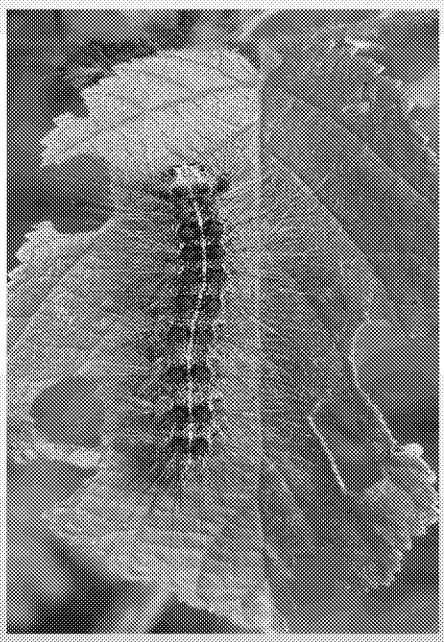
The microbial pesticide, Entomopathogenic fungi, were determined to be the major mortality factor of EAB in wild Michigan populations.
Adult EAB were susceptible to Beauveria bassiana and Metarhizium anisopliae.
Isolate B. bassiana GHA killed EAB adults
Sub-lethal effects: adult longevity reduced from 22 to 13 days in females; 28 to 14 days in males; fewer eggs were produced; EAB larvae took longer to develop.
Beauveria was capable of reducing EAB numbers and slowing ash decline through trunk and foliar applications.
Useful for healthy trees and in core zones slowing progress.

Gypsy Moth: Overview

- Introduced into the U.S. from Europe in 1869
  - Massachusetts businessman hoping to breed the gypsy moth with silkworm
- One of the most invasive insects
- Notorious pests of hardwood trees
- Defoliates 1+ million forested acres annually
- Devours oak, sweetgum, gray and white birch, and poplar



Gypsy moth larva
(Image: Bill McNee; Wisconsin DNR; bugwood.org)

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Gypsy moth was introduced into the U.S. from Europe in 1869 by a Mass. businessman hoping to breed the gypsy moth with the silkworm.
The gypsy moth is one of the most invasive insects, as identified by the World Conservation Union.
It is one of the most notorious pests of hardwood trees - defoliating a million or more forested acres each year.
Particularly susceptible are stands of oak, sweetgum, gray and white birch, and poplar.

Gypsy Moth: Natural Enemies

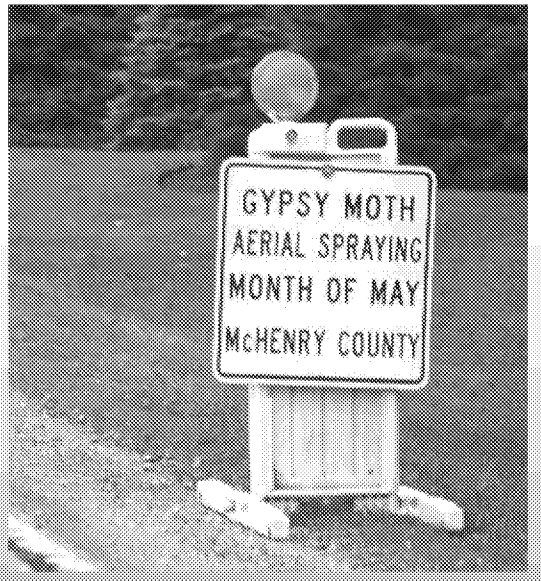
- Two wasps and two flies lay their eggs in gypsy moth caterpillars
  - Larvae feed on the caterpillars ultimately killing their hosts
- Predators include ground beetles, ants, nematodes, birds, and small mammals
- Other organisms that impact gypsy moth larvae including a bacterium, fungus, and virus.

Female gypsy moths laying egg masses
(Image: John H. Ghent, USDA Forest Service; Bugwood.org)

Natural parasitoids of the gypsy moth include two wasps and two flies, each of which lays its eggs in the caterpillars
The larvae feed on the gypsy moth caterpillar ultimately killing their hosts.
Predators of the gypsy moth include ground beetles, ants, nematodes, birds, and small mammals.
several organisms are now being successfully used to control gypsy moth larvae including a bacterium, fungus, and virus.

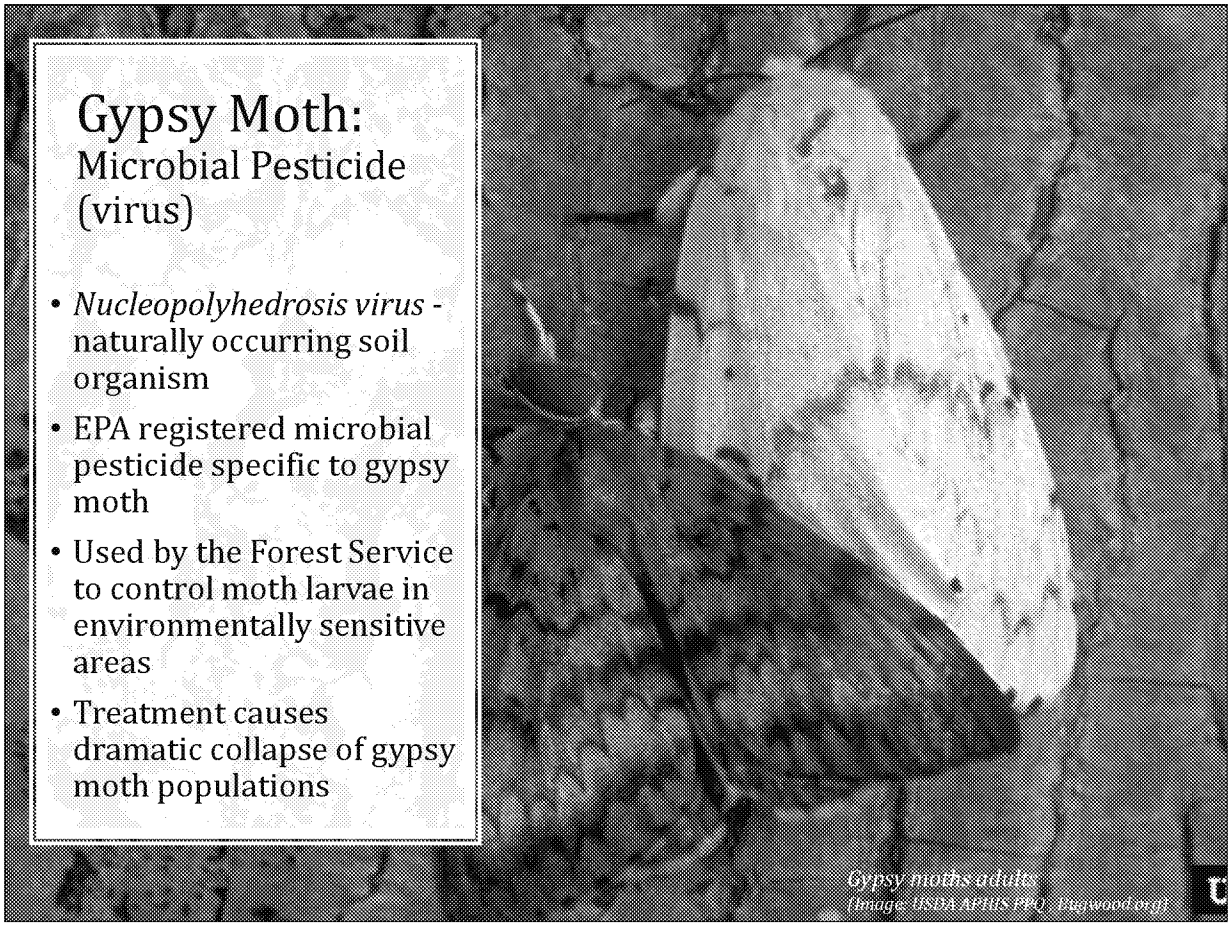
Gypsy Moth: Microbial Pesticide (bacterium)

- *Bacillus thuringiensis* (*Bt*) - naturally-occurring soil bacterium
- EPA-registered microbial pesticide that must be eaten by insect larvae to be effective
- Good spray coverage is essential
- After ingesting *Bt*, the larvae stop feeding and die within a few days.



Sign notifying residents of gypsy moth spraying.
(Image: mchenrycountyblog.com)

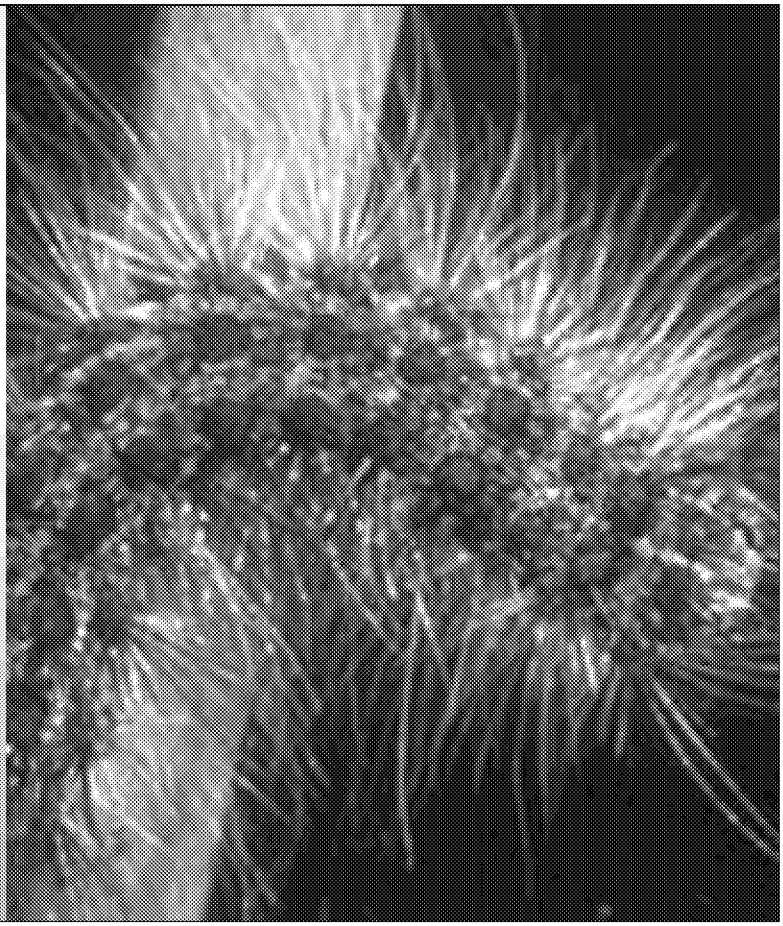
Bacillus thuringiensis (Bt), is a naturally-occurring soil bacterium that has become an important tool in many IPM programs.
Bt is a microbial pesticide that must be eaten by insect larvae to be effective.
After ingesting Bt, the larvae stop feeding and die within a few days.
Because Bt must be eaten to work, good spray coverage of the plant leaves is essential for control.
Bt used for controlling gypsy moth has no effect on other types of insects (such as bees) except for other moth and caterpillar larvae that eat the treated leaves. It is also considered to be "practically nontoxic".



The Lymantria dispar nucleopolyhedrosis virus (NPV) is a naturally occurring organism that persists in the soil.
It has been developed as an EPA-registered microbial pesticide specific to the gypsy moth.
Used by the Forest Service to control gypsy moth larvae in environmentally sensitive areas.
The virus is also called 'wilt' and has the ability to causes a dramatic collapse of gypsy moths when their populations are high.

Gypsy Moth:
Microbial Pesticide (virus) (cont.)

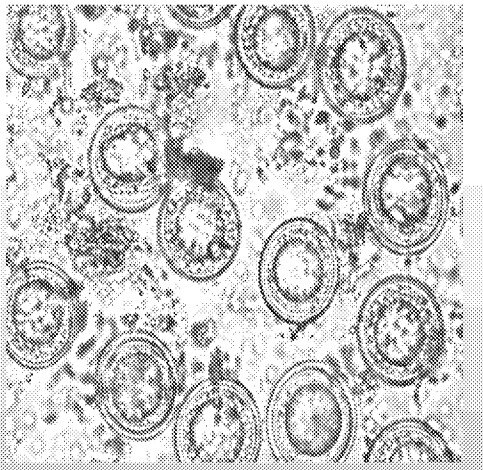
- Caterpillars pick up the virus when eating treated foliage
- Gut dissolves the occlusion bodies and the virus penetrates through gut wall
- Liquefies caterpillars' organs
- Death 10-14 days after infection



NPV liquefies moths' organs.
Gypsy moths 'catch' the virus when they eat foliage contaminated with viral occlusion bodies which contain the particles.
The alkaline condition of the gypsy moth gut dissolves the occlusion bodies and the virus particles penetrate through the gut wall.
The virus reproduces rapidly, quickly liquefies the caterpillars' internal organs, causing death.
Once the gypsy moth larvae eat foliage with the virus on the surface, they die within 10–14 days.

Gypsy Moth: Microbial Pesticide (fungus)

- *Entomophaga maimaiga* – EPA registered microbial pesticide
- Successful in controlling gypsy moths in eastern U.S.
- Caterpillars infected when contacting the fungus on the ground as they move between trees
- Fungal spores actively shoot out of the dead larvae, disperse into the environment, and spread to other caterpillars

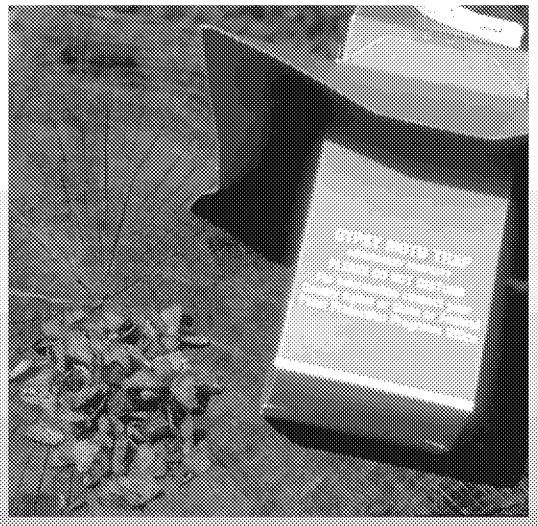


Entomophaga maimaiga spores from a gypsy moth caterpillar
(Image: Tawny Simisky, ag.umass.edu)

A fungal pathogen, Entomophaga maimaiga, has been successful in controlling gypsy moth populations in the eastern U.S.
The caterpillars become infected when they contact the fungus on the ground as they crawl from tree to tree.
Fungal spores actively shoot out of the dead larvae, disperse into the environment, and spread quickly to other caterpillars.

Gypsy Moth: Biochemical Pesticide (pheromone)

- Female gypsy moths do not fly and attract mates by emitting a pheromone
- EPA-registered imitation pheromones
  - Confuse males searching for females and effectively disrupt mating
  - Used in traps to monitor populations

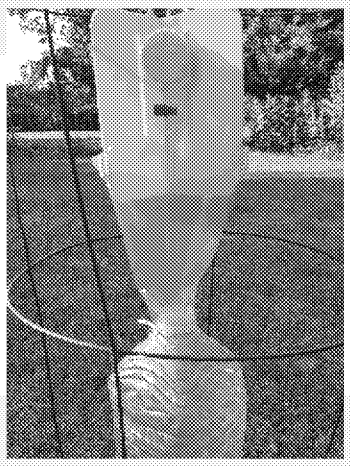


Gypsy moth pheromone trap
(Image: William A. Carothers, USDA Forest Service, Bugwood.org)

Pheromones are yet another group of biopesticides that are important in monitoring and controlling several pests. Female gypsy moths do not fly so they must attract male moths by emitting a pheromone.
Synthesized EPA-registered imitation pheromones are effective in disrupting mating. When pheromones are applied throughout an area, the male moths are confused by the multitude of aerial plumes.

Pheromones

- Sex pheromones are emitted by female insects to attract mates
- Aggregation pheromones are produced by male insects to attract other males, females (adults and larvae)
- EPA-registered as biopesticides that can be used to detect, monitor, and trap insects
- Effective controlling populations by disrupting mating and trapping
- Examples: Japanese beetle and gypsy moth



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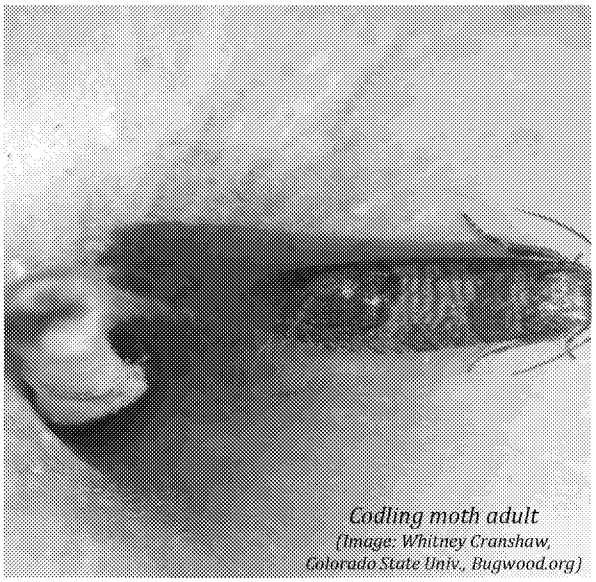
Yes, pheromones are also a bio-pesticide.
Sex pheromones are emitted by female insects to attract mates
Aggregation pheromones are produced by male insects to attract other males, females (adults and larvae)
EPA-registered as biopesticides that can be used to detect, monitor, and trap insects
Pheromones of certain pest insect species, such as the Japanese beetle and gypsy moth, can be used to trap the respective insect for monitoring purposes, to control the population by disrupting mating or through mass trapping.

Codling Moth: Microbial Pesticide (virus)

- Codling moth larvae burrow into fruit rendering it unsellable
- Codling moth has some resistance to traditional insecticides
- Entomopathogenic *Cydia pomonella* granulovirus
  - natural pathogen of codling moth larvae
  - EPA registered microbial pesticide
  - No observed resistance



Codling moth adult
(Image: Whitney Cranshaw, Colorado State Univ., Bugwood.org)

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The codling moth is a problematic pest on several fruit trees including apples and pears.
Codling moth larvae burrow into fruit, rendering it unsellable.
A viral biopesticide, CpGV (Cydia pomonella granulovirus), is a natural pathogen of codling moth larvae.
It is registered by EPA as a microbial pesticide.
There has been no observed resistance to CpGV in the codling moth, which is prone to develop resistance to many pesticides.

Root Rot: Microbial Pesticide

- *Phlebiopsis gigantea* strain VRA 1992
- EPA-registered microbial pesticide to control pathogenic root rot and butt rot on conifers caused by *Heterobasidion annosum*
- *P. gigantean* is a widely-distributed, naturally occurring wood-decay fungus found throughout North American boreal and temperate forests
- The biopesticide has low toxicity and highly specific to target pest
- Alternative to sodium tetraborate or sodium octaborate

H. annosum root rot
(Image: Andrej Kunca, National Forest Centre - Slovakia, Bugwood.org)

Still another example of microbial pesticides that can be of use in the tree industry revolves around root rot.
Phlebiopsis gigantea strain VRA 1992 is EPA-registered to control root rot and butt rot (Heterobasidion annosum complex) on conifers.
The pesticide is used in thinned pine forests to prevent the infection of cut stumps which can spread rot through their roots to adjacent, healthy trees.
When applied to a freshly-cut stump, the microbe will grow into the wood excluding rot fungi that can damage healthy trees.
It serves as a reduced risk alternative to sodium tetraborate decahydrate and sodium octaborate tetrahydrate.

Through these many examples of pest issues that you probably deal with on a regular basis, I hope you can see that biopesticides can be a part of your pest control toolbox.
As part of an integrated pest management program, EPA-registered biopesticides can aid the arborist and forestry industry in insuring the health of our nation's trees.

I'd like to thank the New Jersey Arborists Chapter of the ISA for the opportunity to speak with you all today.
I'm happy to take your questions.