CTPB Final Research Report

Project Number: 20-10-WVU
Project Title: Isolation and development of effective fungal biocontrol for elongate hemlock scale
Principal Investigator: Matt T. Kasson, PhD
Institution: West Virginia University Division of Plant and Soil Sciences

1. Technical report

Introduction

Elongate hemlock scale (Fiorinia externa Ferris; EHS) is an armored scale insect pest of Christmas tree nurseries. This insect was introduced into the United States in the early 1900's from Japan. In the Eastern United States (i.e., Northeastern, mid-Atlantic and Midwest), EHS is unchecked by its natural enemies and contributes to the decline of native eastern and Carolina hemlock across forested ecosystems in the eastern United States (Figure 1, McClure, 1986). In Christmas tree operations, the development of biopesticides to control EHS has been slow. Biopesticides that have been developed for EHS have been found to live endophytically and/or to be plant pathogens (Marcelino 2007; Marcelino et al., 2009). This presents challenges for their development that could be circumvented with a specialized EHS pathogen. In this project, we sought to identify fungal pathogens of EHS that were causing natural epizootics in Christmas tree orchards.

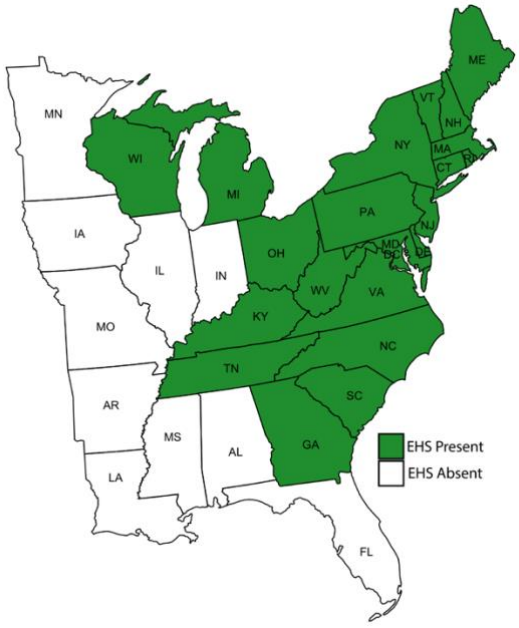

Figure 1: Range of elongate hemlock scale across the eastern United States.

Methods

Scouting for natural EHS fungal pathogens

In August of 2020, Dr. Matt Kasson (principal investigator) and Dr. Brian Lovett (project leader) traveled to Ashe County North Carolina to scout for fungi causing EHS epizootics. This travel was critical for the success of this project, but every effort was made to follow stringent COVID-19 guidelines from the CDC and WVU, including mask use and a 5-day quarantine following interstate travel. During this trip, five sites were surveyed: Vannoy Farm, Deal Family Farm, Upper Mountain Research Station, Mount Rogers Seed Orchard and Mount Rogers. These sites spanned throughout Grayson County Virginia and Ashe County North Carolina. At the recommendation of our collaborators, we intentionally visited these sites because a majority had high levels of EHS and minimal management, and thus more opportunity for outbreaks of fungal pathogens. Branches were examined visually using a 15X magnification hand lens to confirm mycosis due to fungi. All sites

with fungus infected EHS cadavers were sampled and brought back to the lab for further processing.

Isolation of fungal pathogens from EHS cadavers

Needles that had EHS cadavers with visible mycosis were identified and individually isolated for each site. Under a biosafety cabinet, we removed cadavers using a sterile scalpel and placed the entire insect and fungal outgrowth onto petri dishes containing potato dextrose agar supplemented with streptomycin and tetracycline (PDA+ST). These plates were maintained at room temperature until fungal growth was observed. Colonies with morphology matching the observed mycosis were isolated using standard microbiological techniques. Up to 5 strains from each site were isolated, and all isolated EHS pathogens were stored on PDA slants for long-term preservation.

Needle endophytes

We assessed the fungal community associated with EHS and the most prevalent fungal pathogen of EHS (*Conoideocrella luteorostrata*, CL) by isolating fungi from needles pulled with the following statuses: EHS positive, EHS and CL positive, or EHS negative. From multiple sites (when available), needles with these statuses were surface sterilized using a bleach solution. In triplicate, four needles with each status were then placed on PDA+ST. Daily, these plates were observed, and all unique fungal morphotypes were isolated. This process was continued until fungi covered the plate: preventing emergence of other morphotypes. All isolated needle endophytes were stored on PDA slants for long-term preservation.

Phylogenetics

We conducted a phylogenetic study to assess the relationships among recovered strains as well as their relationships with reference DNA sequences deposited in NCBI GenBank, an NIH public repository for fungal DNA sequence data. We targeted sequencing of the fungal barcoding gene, the internal transcribed spacer region (ITS), D1–D2 domains of nuclear 28S rRNA gene (28S), and elongation factor 1 alpha (EF1-α). Single-gene and concatenated phylogenetic trees were constructed for representative strains recovered in this survey, along with additional reference sequences available from NCBI GenBank.

Laboratory bioassays

We have optimized a bioassay protocol to infect EHS with CL directly. This will refine our understanding of infection in crawlers: the life stage most vulnerable to CL according to our field observations. These bioassays involve the collection of local hemlock branches with EHS adult females and eggs (resting under adult female tests) and rearing crawlers in the lab. We placed infested 5-cm branch tips onto petri dishes placed on top of a dampened paper towel to provide appropriate humidity for hatching and maintaining the plant host. Crawlers that have emerged in batches over 5-7 days are used for experiments, which allows standardized time of treatment. Crawlers are introduced using a paintbrush to fresh plant material using a paintbrush, to remove any other life stages and streamline observation. Once settled, these branches with settled crawlers are dipped into a spore suspension of $1x10^7$ CL spores in 0.01% Tween20. Survival of

EHS treated with fungus or the same solution without fungus (as a negative control) was observed daily under full magnification of a dissecting scope.

Results

Isolation of fungal pathogens from EHS cadavers

In 2020, we successfully scouted for, recovered, and identified a potential biocontrol fungus of EHS, *Conoideocrella luteorostrata,* from Ashe County, North Carolina. This specialized armored-scale pathogen was common across all infested orchards and representative strains were retained in pure culture for subsequent morphological and DNA-based studies and for bioassays to evaluate pathogenicity and efficacy.

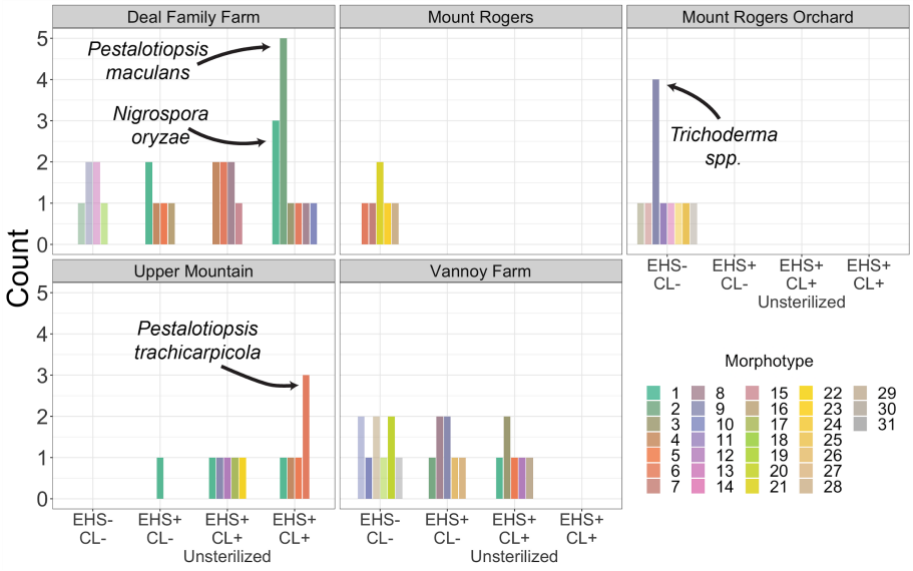

Figure 2: The distribution of fungal morphotypes across sites for sampled needles from each condition. "CL" represents *Conoideocrella luteorostrata* and "EHS" represents elongate hemlock scale.

Needle endophytes

Despite *Conoideocrella luteorostrata* being widespread in our samples, repeated attempts to isolate this fungus from surface-sterilized needles harboring healthy and/or mycosed EHS, as well as EHS-free needles, failed to recover this fungus, supporting its role as an entomopathogen that lacks a facultative endophytic lifestyle. Numerous fungi were recovered across all needle categories, including common needles inhabitants such as *Pestalotiopsis* spp. (Figure 2). Interestingly, an unidentified *Colletotrichum* sp., which was previously identified as a facultative entomopathogen of EHS, was also recovered from needles. This emphasizes the endophytic niche that *Colletotrichum* spp. may occupy upon inundative application.

Phylogenetics

Due to insufficient reference sequence data available in NCBI GenBank, only EF1 sequence data were used for phylogenetic analysis until we can obtain physical cultures of isolates from Japan and Thailand to generate additional sequence data to permit proper comparisons. The results of our analysis indicate that all isolates recovered across multiple sites in North Carolina were near-identical and formed a clade with isolates from Japan and Thailand (Figure 3). Some sequence divergence (e.g., VF 1-1) may reflect actual small differences in sequence data or may simply be low-quality sequences that need to be re-sequenced. The lack of support for the whole clade may suggest that isolates from Thailand are a separate species (based on strong

bootstrap support for this group). Reliance on a single gene for evaluating these genetic relationships is insufficient to distinguish this potential sister relationship. A multi-gene approach will undoubtedly help to resolve these relationships.

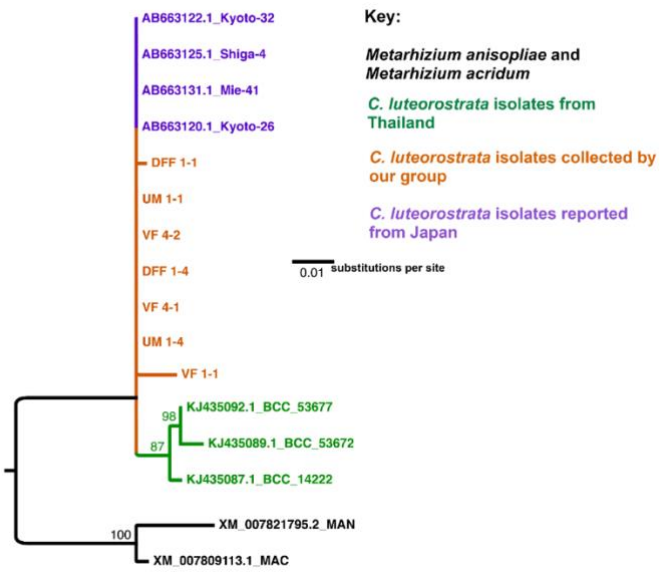

Figure 3: The genetic relationships among *Conoideocrella luteorostrata* strains recovered from EHS in Japan and North Carolina and from whitefly nymphs in Thailand.

Laboratory bioassays

We commenced bioassays in Spring 2021 (following isolation of CL). Our early efforts focused on providing laboratory conditions that could sustain EHS crawlers, which easily dry out, while preventing growth of contaminating fungi. Crawlers emerge quickly, and we found that a moistened towel placed under plates that contain branches could sustain crawlers. A challenge for our bioassays is that EHS crawlers emerge asynchronously. This means that treating samples that were collected directly from the field results in increasing numbers of individuals. To address this, we developed a method to transfer a set number of freshly emerged crawlers onto uninfested hemlock branches using a paintbrush. Attempts to maintain crawlers on noble agar led to early death and growth from contaminating fungi. Once settled on branches, crawlers survive dipping into a spore suspension containing 0.01% Tween20 and CL conidia. This bioassay method was used to infect field collected EHS with CL, which we confirmed by surface sterilizing multiple cadavers and re-isolating CL from within the insect on PDA. We also observed discrete stages of infection during this bioassay (Figure 4).

Figure 4: Dissecting scope images of EHS crawlers during laboratory bioassays. These each are representative of distinct phases of CL infection from asymptomatic “normal” individuals to mycosis (when fungus grows out of the insect and sporulates).

Discussion

In the course of these studies, we isolated a promising biocontrol agent for controlling EHS that we identified morphologically and molecularly as CL. This fungus was observed to naturally control EHS in endemic Christmas tree orchards in Ashe County North Carolina. Given its limited geographic range, near clonal genetic structure, and efficacy against EHS, preliminary data indicate that these strains of CL likely coevolved alongside EHS. This possibly occurred in

Japan or elsewhere in east Asia, and this pathogen was subsequently introduced into the eastern United states. Future efforts will aim to delimit the range of this fungus and determine its incidence on hemlock and fir hosts in the central Appalachian region. Yet even with these uncertainties, needle isolation data indicates that *C. luteorostrata,* unlike many of the previously proposed fungal biocontrol agents for this invasive pest, does not persist endophytically in EHS infested and/or healthy conifer needles or twigs. This suggests that the biology of this fungus is limited to attacking and killing EHS, and observed natural epizootics suggest successful application may continue to control the population over time.

Future studies related to fungal biology will focus on further resolving the genetic relationships among strains from Japan, Thailand, and the United States. Furthermore, mating type studies are needed to determine the thallism of populations present in the United States and determine if both mating types are present, which would allow for sexual reproduction. We have thus far only observed asexual structures (i.e., no fruiting bodies and only conidia) from CL in the United States, suggesting this population reproduces clonally.

More in-depth bioassays are needed to understand the process of infection, to evaluate persistence of the conidia and to optimize rearing of EHS in the lab for evaluation. It is clear that CL infects EHS on contact, as is typical for a specialized insect pathogen. This mode of infection is different from other potential biocontrol fungi for EHS that infect opportunistically as endophytes (Li et al., 2017; Marcelino et al., 2017), so comparative bioassays among these strains may not be possible or informative. Methods developed for bioassays using CL could be easily adapted to test other interventions to control EHS or other scale insects.

Our work to develop CL as a biocontrol fungus marks an exciting new chapter for EHS management. Our results thus far suggest that this entomopathogen has enormous potential to control EHS specifically, to be developed commercially and to be applied successfully in Christmas tree settings.

References

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2. Summary of Research Report for Public Release by CTPB

Elongate hemlock scale (EHS) completes its life cycle on the underside of needles of primarily fir and hemlock. This insect threatens Christmas tree production in the Eastern United States by impacting tree health, aesthetic value and exportability. These characteristics are paramount for a salable Christmas tree, and so this very small insect (~2 mm long) is a large concern for growers. Unfortunately, EHS may evolve to resist chemical pesticides. Moreover, these pesticides can also kill insects that are natural enemies of EHS, which can ultimately worsen EHS infestations.

In our Christmas Tree Protection Board (CTPB) funded project, we sought to identify fungi that naturally infect and control EHS in Christmas tree operations. After scouting in Ashe County North Carolina in 2020, an insect-killing fungus with a high degree of specificity was found to be present across multiple Christmas tree orchards. Multiple strains of this fungus were isolated in the lab and identified as *Conoideocrella luteorostrata* (Figure 1A). This fungus kills armored scale insects, including EHS, in its native range in southeastern Asia. Importantly, the narrow host range of this fungus suggests that it would not infect natural enemies of EHS. Our work revealed that the same fungus is naturally causing outbreaks of disease (killing immature EHS) in North Carolina. We sought to further characterize this fungus to ultimately develop methods to apply this fungus directly to Christmas trees to control EHS.

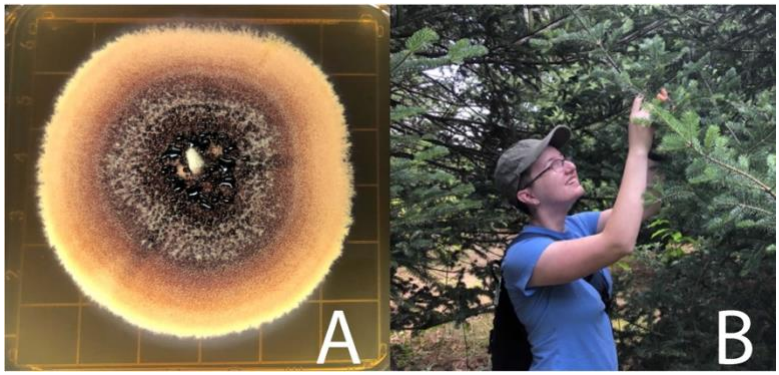

Figure 1: A) *Conoideocrella luteorostrata* isolated on a petri dish in the lab. **B)** West Virginia University undergraduate student Hosanna Barrett scouting for EHS and CL at a Christmas tree orchard in Bruceton Mills, West Virginia.

Other fungi, such as *Colletotrichum fioriniae* and *Metarhiziopsis microspora,* have been developed for this purpose; however, the potential application of these fungi is complicated by their ability to live within needles or infect other plants. We sought to isolate CL from within the needles of sampled Christmas trees, including those with EHS cadavers colonized with CL, to see if CL adopted a similar lifestyle within plant tissue. These experiments revealed that CL is naturally limited to EHS in Christmas tree operations. This is an ideal characteristic for a potential biocontrol agent. During our project, we have worked to develop bioassays to challenge EHS with CL and observe the infection process. This will allow us to evaluate the efficacy of this fungus for development into a commercial biopesticide.

Aside from the important research milestones we accomplished during this project, it also provided a formative training opportunity for Hosanna Barrett (Figure 1B), an undergraduate student at West Virginia University (WVU). For this student, this project has led to a WVU Summer Undergraduate Research Experience, multiple presentations at conferences and an honors undergraduate thesis describing the biology of CL in detail.

3. List of all Publications related to this Research Grant

Publications

- WVU Extension fact sheet entitled “Elongate hemlock scale in the eastern United States” submitted and awaiting release (**attached**)
    - English and Spanish versions were submitted
- Projected publication on the isolation of *Conoideocrella luteorostrata* from eastern United States, phylogenetics on isolated strains and results from bioassays in *Journal of Integrated Pest Management*, *Mycologia* or similar by 2022.

Oral abstracts

- WVU Undergraduate Research Symposium 29 July 2021
- Undergraduate Research Day at the Capitol 5 March 2021

Poster abstracts

- Mycological Society of America: Botany 2021 (**attached**) 21 July 2021
    - MSA Undergraduate Poster Presentation Award Winner
- WVU Undergraduate Research Symposium 17 April 2021
- American Phytopathological Society Potomac Division Meeting 11 March 2021

Submitted to WVU Extension in June 2021

Elongate hemlock scale in the eastern United States

Introduction

Elongate hemlock scale (*Fiorinia externa*) is an insect pest of several species of coniferous trees. They are native to Japan and China, but have become widespread in the eastern United States. In the US scale populations tend to be denser and have a greater impact on the host than in their native range where synchronized natural predators prevent high infestations (McClure 1986). They are of particular concern to Christmas tree farmers because infestations are unsightly and can cause symptoms including yellowing (chlorosis; Figure 1), needle drop, and occasionally tree death (Van Driesche et. al. 2013). They also present issues concerning export and import of trees.

Figure 1: Mottled yellowing on the top of hemlock needles (inset) corresponding with elongate hemlock scales on the underside.

Description

Elongate hemlock scale is an armored scale insect that lives on the underside of needles, particularly on hemlock, fir and pine. These insects have multiple stages which are barely visible to the eye (Figure 2). A single needle can contain many individuals of this scale insect all in different stages. Beginning in their nymphal stages, female adult elongate hemlock scales are immobile and secrete a brown, waxy covering with fades to yellow. The covering is oval shaped and about 2 mm long. Females will lay eggs underneath their own covering, and these rows can be visible through the covering. Immature nymphs are yellow and dorsoventrally flattened. Males produce a white waxy cover with the insect visible as a yellow tip and are about 1.5 mm long (smaller than females). Adult males live only a short while and are small and brown with a single pair of wings.

- Female adults are soft bodied, legless, wingless, and under a waxy cover that is light brown and about 2mm long.
- Male adults are light brown,1.5mm long, have legs and wings that fly feebly.
- Crawlers are soft bodied, legged, and 0.1mm to 1.0mm.

Figure 2: EHS eggs overwinter under the protective waxy covering of their mother. Eggs hatch into crawlers which become immobile indefinitely for females and until adulthood for weakly-flying males. A natural outbreak of the insect-killing fungus *Conoideocrella luteorostrata* in elongate hemlock scale on Fraser fir is also shown. Egg photo credit to Pennsylvania Department of Conservation and Natural Resources, adult male photo credit to Pennsylvania Department of Agriculture and crawler photo credit to Eric. R. Day at Virginia Polytechnic Institute.

Biology and Life Cycle

Elongate hemlock scales begin their life as a group of light-colored eggs protected under an oval-shaped waxy cover. When the weather warms seasonally around May, their eggs hatch to emerge as soft-bodied, legged crawlers which are about 0.1 mm long and lemon-colored. Crawlers are the only mobile life stage, and the primary life stage that spreads to new plants. Since scales have staggered reproductive cycles across the year, more than one stage can be observed at the same time (Kosztarab 1996). They can be carried by the wind or birds. Adult males are about 1.5mm long, light-colored, and have visible legs and wings. They are mobile but weak fliers. The most visible stage is the adult female, which is around 2mm long, covered in a lemon-colored to brown waxy cover, and is immobile.

Hosts and distribution

Scales are most commonly found on eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*T. caroliniana*) but have been observed on many species including spruce (*Picea*), fir (*Abies)*, and yew (*Taxus)* (McClure and Fergione 1977). In Christmas tree operations, this is a pest of true fir (*Abies* spp.) that can also be found on Douglas fir (*Pseudotsuga menziesii*), spruce and pine trees (Sidebottom 2016).

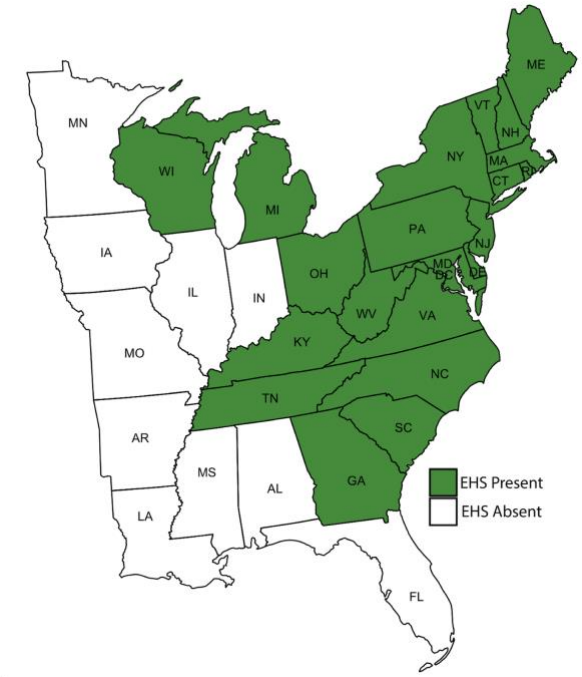

Figure 3: State-level elongate hemlock scale range in 2021 compiled from available reports.

EHS was first reported in the United States from New York in 1908: it was likely imported from Japan (Sasscer 1912). Since, this insect has spread through 22 states along the eastern United States (Figure 3).

Management

Integrated pest management, IPM, uses a combination of a variety of techniques to keep the population of a pest below a reasonable limit. If we identify the pest and use our knowledge of its lifestyle to deploy a variety of control techniques, we can establish a balance which maintains plant health in the long term with limited negative side effects. The main components of IPM are identification and evaluation of the issue, actions to reduce pests and prevent further outbreaks, and long term monitoring to adjust the strategy as necessary. Below are some strategies which can be integrated into a pest management system for elongate hemlock scale.

Cultural Control

Provide plants with good growing conditions (e.g. spacing) and proper cultural care (e.g. proper pruning). Appropriate irrigation reduces stress and increases tolerance to scale insect damage. Also, over-fertilization can increase scale reproduction resulting in higher scale densities (McClure 1980).

Biological control

Several natural enemies of hemlock scales are present under natural conditions. For example, the cosmopolitan parasitoid wasp *Encarsia citrina* (Figure 4) regularly kills 90% of the hemlock scale population in Japan and is well established in the United States (Abell and Van Driesche 2011). The predatory beetle, *Cybocephalus nipponicus* (Figure 4), also preys on these scales in their range in the United States (Mayer et. al. 2008). In addition, generalist predators such as lady beetles and lacewings are commonly found on coniferous trees. Lady beetles, lacewings, *E. citrina* and other natural enemies of scale insects are also commercially available. Lastly, entomopathogenic fungi, such as *Colletotrichum fioriniae* (Marcelino et al., 2008) and *Conoideocrella luteorostrata*, can infect and kill nymphs and adult scales (Figure 2).

Figure 4: Two natural enemies of EHS are the small black beetle *Cybocephalus nipponicus* (left; males have a yellowish pronotum) and the parasitoid wasp *Encarsia citrina* (right; shown parasitizing an elongate hemlock scale female). Beetle photo credit to Pennsylvania Department of Conservation and Natural Resources.

Chemical control

Timing of the application is important for effective chemical control because scale insects become less susceptible to insecticides as they age. Several insecticides with different active ingredients kill armored scales when targeting at the crawler stage. Horticultural oils and reduced-risk insecticides, such as buprofezin, pyriproxyfen and spirotetramat, are recommended because of their efficacy and compatibility with beneficial insects. Other active ingredients, such as bifenthrin, imidacloprid, dinotefuran and acephate, also kill armored scales; however, they are harmful to beneficial organisms and can cause spider mite outbreaks. Many products are commercially available with the mentioned active ingredients but federal laws indicate that the site of application must be listed on the pesticide label.

Written by Hosanna Barrett, Dr. Brian Lovett, Dr. Matt Kasson and Dr. Carlos Quesada at West Virginia University.

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La Escama del Falso Abeto

Introducción

La escama del falso abeto (*Fiorinia externa)* es una plaga de varias especies de plantas coníferas. También es conocida como cochinilla o EHS (por sus siglas en inglés, enlogate hemlock scale). Esta escama es nativa de Japón y China, pero se ha establecido en el noreste de los Estados Unidos. En los Estado Unidos, poblaciones de esta escama son más densas y tienen un mayor impacto en las plantas que en su área de origen, donde sus depredadores naturales previenen altas infestaciones (McClure 1986). La escama del falso abeto causa preocupación a los productores de árboles de Navidad porque sus infestaciones causan mal aspecto en estos árboles por sus síntomas que incluyen coloración amarillenta (clorosis; Figura 1), caída de las hojas y ocasionalmente la muerte de los árboles (Van Driesche et. al. 2013). También ocasiona problemas relacionados con la exportación e importación de árboles.

Figura 1: Coloración amarillenta en la parte superior de las hojas del falso abeto (hemlock) causado por las escamas en la parte inferior.

Descripción

La escama del falso abeto es un insecto que vive en la parte inferior de las hojas de los árboles, particularmente en pinos, abetos y falsos abetos. Una sola hoja puede contener muchas escamas en sus diferentes etapas. Las hembras en su etapa ninfal son inmóviles y segregan una cubierta cerosa de color marrón a amarillo. La cubierta es de forma ovalada y de aproximadamente 2 mm

de largo. Las hembras ponen huevos debajo de su propia cubierta los cuales pueden ser visibles a través de la misma. Las ninfas son amarillas y dorsiventralmente aplanadas. Los machos producen una cubierta cerosa blanca con una punta amarilla y miden aproximadamente 1.5 mm de largo (son más pequeños que las hembras). Los machos viven poco tiempo, son pequeños y de color marrón con un par de alas en su etapa adulta.

- Las hembras tienen cuerpo blando, sin patas ni alas y forman una cubierta cerosa de color marrón claro de aproximadamente 2 mm de largo.
- Los machos son de color marrón claro, miden 1.5 mm de largo, tienen patas, alas y vuelan con dificultad.
- Las ninfas son móviles por las primeras 48 horas de vida, tienen cuerpos bando, patas y miden 0.1 a 1.0 mm.

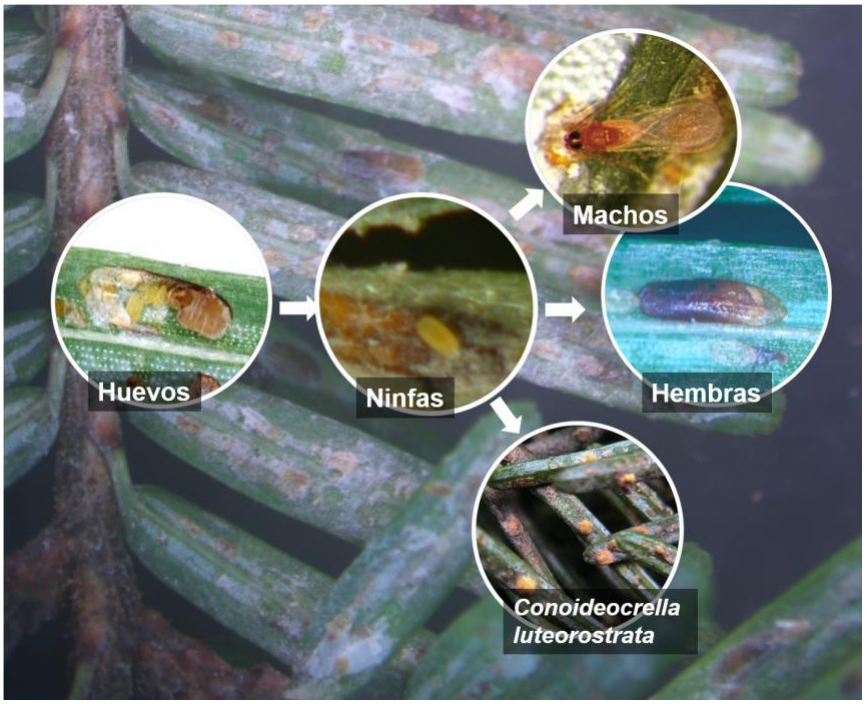

Figure 2. Los huevos de las escamas pasan el invierno bajo la cubierta cerosa de su madre. Las ninfas son móviles al salir del cascaron y se vuelven inmóviles rápidamente. Las hembras nunca recuperan movilidad mientras que los machos vuelan con dificultan en su etapa adulta. También se muestra un brote natural del hongo *Conoideocrella luteorostrata* que mata escamas en el abeto. La foto de los huevos fue tomada por el Departamento de Conservación y Recursos Naturales de Pensilvania, la foto del macho por el Departamento de Agricultura de Pensilvania y la foto la ninfa por Eric. R. Day en el Instituto Politécnico de Virginia.

Biología y Ciclo de Vida

Los huevos son de color claro y están protegidos por una cubierta cerosa de forma ovalada. Cuando la época calurosa inicia, alrededor de mayo, los huevos eclosionan en ninfas. Las ninfas miden aproximadamente 0.1 mm de largo, son de color limón y tiene cuerpo blando. Las ninfas son la etapa fundamental para infestar nuevas plantas. Dado que las escamas tienen varios ciclos reproductivos a lo largo del año, se puede observar diferentes etapas al mismo tiempo (Kosztarab 1996). Ninfas pueden ser transportadas por pájaros y el viento. Los machos adultos miden aproximadamente 1.5 mm de largo, son de color claro y tienen alas, pero vuelan con dificultad. La etapa más fácil de observar es la hembra adulta la cual mide alrededor de 2 mm de largo, tiene una cubierta cerosa de color limón a marrón y es inmóvil.

Hospedadores y distribución

Las escamas se encuentran con mayor frecuencia en el falso abeto (*Tsuga canadensis*) y abeto oriental (T. caroliniana), pero se han observado en otros tipos de abetos, pinos (Picea) y el tejo (Taxus) (McClure y Fergione 1977).

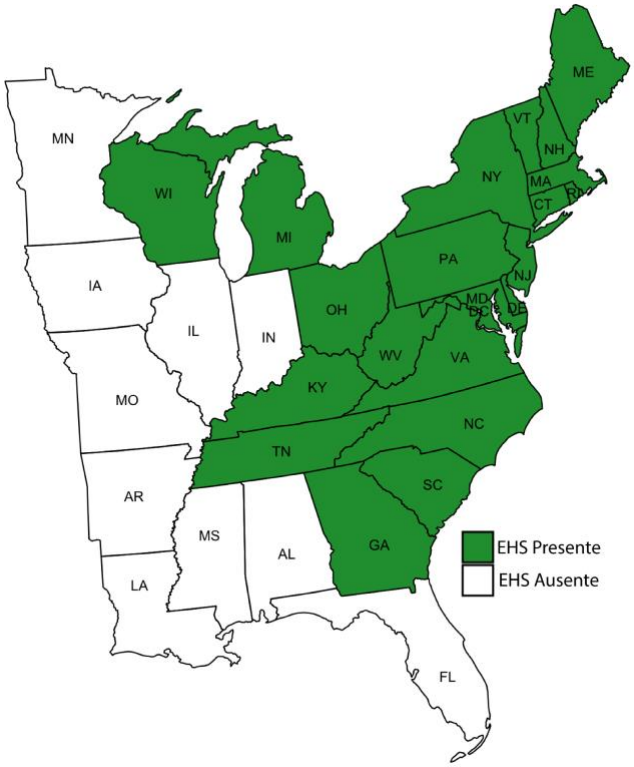

Figura 3. Rango de la escama del falso abeto a nivel estatal en 2021 compilado de reportes disponibles. Los estados en verde indican la presencia de la escama del falso abeto

Manejo

Manejo integrado de plagas, MIP (o IPM, por sus siglas en inglés) es la combinación de varias técnicas para controlar la población de una plaga por debajo de un límite razonable. Si identificamos la plaga y utilizamos lo que se conoce de su comportamiento para implementar técnicas de control, podemos establecer un equilibrio que mantenga la salud de las plantas a largo plazo sin o con pocos efectos secundarios. Los componentes principales del MIP son la identificación del problema, evaluación, las acciones para reducir las plagas y prevenir nuevos brotes. También el monitoreo a largo plazo para ajustar la estrategia según sea necesario. Las siguientes son algunas estrategias que pueden integrarse en un sistema de manejo de plagas para esta escama.

Control cultural

Proporcionar buenas condiciones de crecimiento para las plantas (por ejemplo, suficiente espacio) y un manejo adecuado (por ejemplo, una poda apropiada). El riego reduce el estrés y aumenta la tolerancia al daño causado por las escamas. Además, exceso de fertilizante puede aumentar la reproducción de las escamas, lo que puede resultar en densidades de escamas altas (McClure 1980).

Control Biológico

Muchos enemigos naturales de la escama del falso abeto están presentes en condiciones naturales. Por ejemplo, la avispa parasitoide *Encarsia citrina* (Figura 4) mata regularmente al 90% de la población de insectos en Japón la cual también está establecida en los Estados Unidos (Abell y Van Driesche 2011). El escarabajo depredador, *Cybocephalus nipponicus* (Figura 4), también se alimenta de estas escamas en su área de distribución en los Estados Unidos (Mayer et. Al. 2008). Además, los depredadores como las mariquitas y crisopas se encuentran comúnmente en los árboles coníferos. Las mariquitas, crisopas, *E. citrina* y otros enemigos naturales de las escamas también pueden comprarse para su distribución. Por último, los hongos entomopatógenos (que atacan insectos), como *Colletotrichum fioriniae* (Marcelino et al., 2008) y *Conoideocrella luteorostrata*, pueden matar ninfas y adultas (Figura 2).

Figura 4. Dos enemigos naturales de la escama del falso son el pequeño escarabajo negro *Cybocephalus nipponicus* (izquierda; los machos tienen un tórax amarillento) y la avispa parasitoide *Encarsia citrina* (derecha; se muestra parasitando una hembra alargada de escamas de cicuta). Foto del escarabajo fue tomada por el Departamento de Conservación y Recursos Naturales de Pensilvania.

Control Químico

La época de aplicación es importante para un control efectivo porque las escamas se vuelven menos susceptibles a los insecticidas con el tiempo. Varios insecticidas con diferentes ingredientes activos matan las ninfas en su etapa temprana. Se recomiendan los aceites hortícolas y los insecticidas de bajo riesgo como buprofezin, pyriproxyfen y spirotetramat debido a su efectividad y compatibilidad con insectos beneficiosos. Otros ingredientes activos, como bifenthrin, imidacloprid, dinotefuran y acephate también matan las escamas, sin embargo, pueden afectar a organismos benéficos y causar brotes de ácaros. Hay muchos productos disponibles comercialmente con los ingredientes activos antes mencionados, pero las leyes federales indican que el lugar o cultivo de aplicación debe estar en la etiqueta del pesticida.

Escrito por Hosanna Barrett, Dr. Brian Lovett, Dr. Matt Kasson and Dr. Carlos Quesada en West Virginia University.

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Conoideocrella luteorostrata, a recently discovered entomopathogen and potential biocontrol of elongate hemlock scale in the eastern United States.

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Introduction

Elongate hemlock scales (EHS; Fiorinia externa), are an insect pest of conifers, which cause symptoms including needle yellowing, and needle drop (Figure 1). This is an issue for Christmas tree farmers.[1] EHS eggs overwinter under the protective waxy covering (or test) of their mother. Eggs hatch into crawlers (nymphs) which become immobile indefinitely for females or until adulthood for males (Figure 2).[2] EHS is native to Southeast Asia but has become widespread in the Northeastern United States (Figure 3).[1]

Figure 1: Symptoms of EHS infection on hemlock.

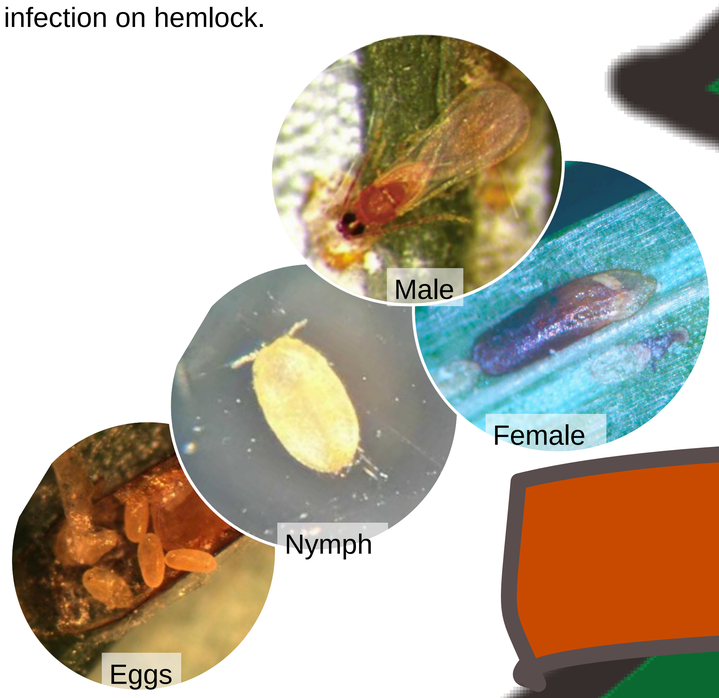

Figure 2: EHS lifecycle.

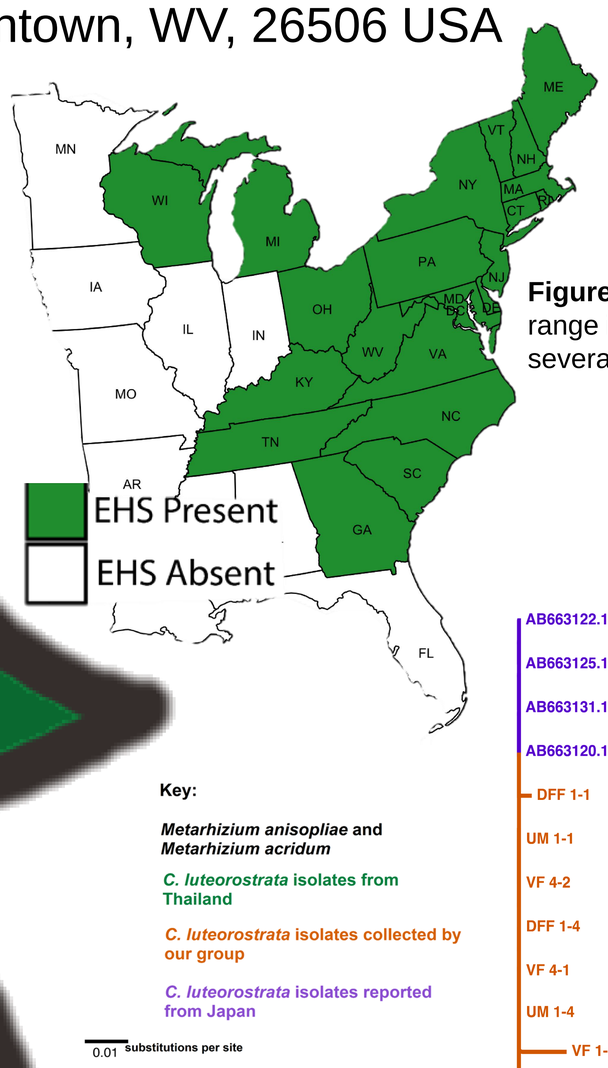

Figure 3: EHS range in 2021 from several sources.

Aim:

Investigate natural fungal pathogens of elongate hemlock scale in the Eastern U.S. for development as biological control agents.

Methods and Results

Collection and Identification:

- An orange fungus causing an epizootic among EHS was found in EHS populations on Fraser fir in Ashe County North Carolina and Grayson County Virginia.
- This fungus was isolated from multiple cadavers from each site.
- The fungus was not isolated from surface sterilized needles.
- This fungus was identified through genetic sequencing as *Conoideocrella luteorostrata*, which is known to infect such scales in its native range in Asia.[3] This was supported by the morphology of our cultures macroscopically, microscopically, and on EHS cadavers.[4]
- The fungus was not isolated from surface sterilized needles.

Infection of EHS

- Branches with EHS crawlers were dipped in a CL spore solution and maintained at 70 C with a damp paper towel to elevate the humidity.
- CL was isolated from a dead crawler 3 weeks after treatment.

Results and discussion

Phylogenetics:

A phylogenetic tree of elongation factor 1-α (EF1-α) sequences from our *C. luteorostrata* isolates and other sequences deposited in NCBI revealed that our *C. luteorostrata* strains are indistinguishable from one another and isolates from Japan, whereas the Thai isolates form their own clade within the species. This provides support for our hypothesis that this epizootic is caused by a clonal population.

Figure 4 shows a phylogenetic tree of the EF1-α genetic sequences from several of our *C. luteorostrata* isolates and other sequences deposited in NCBI (1000 bootstraps).

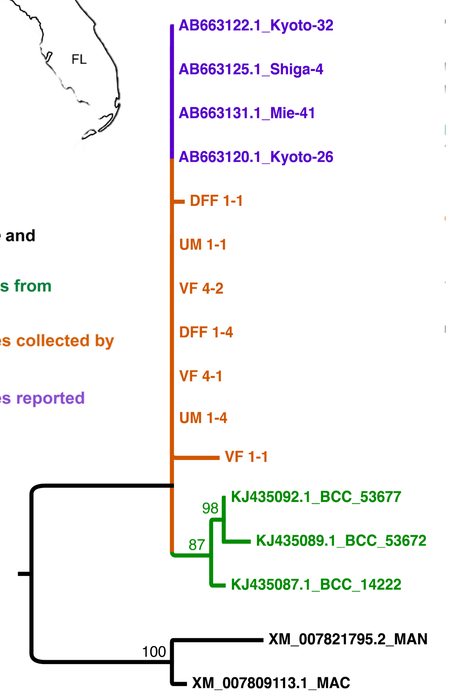

Figure 4: Genetic alignment of our fungus with other strains.

Next steps

In order to prepare *C. luteorostrata* for formulation as a biocontrol we will:

1. Construct phylogeny and diversity of *C. luteorostrata* population in sampled region.
2. Observe the infection process of *C. luteorostrata* in *Fiorinia externa* and whiteflies, or *Trialeurodes vaporariorum*.
3. Conduct bioassays challenging insect pests (*Fiorinia externa* and *Trialeurodes vaporariorum*) and natural predators (*Cybocephalus nipponicus* and *Encarsia citrina*) with *C. luteorostrata*.

This work was done on the traditional land of the Cherokee, Yuchi, Moneton, Osage, Shawnee, and Massawomeck peoples.

Hosanna's research was facilitated by WVU's honors Biology capstone program, under Dr. Susan Raylman in the WVU Department of Biology, and Summer Undergraduate Research Experience, under Dr. Michelle Richards-Babb. This project was funded by the Christmas Tree Promotion Board Grant #20-10-WVU.

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