Classical Biological Control of the Elongate Hemlock Scale, *Fiorinia externa*: 2004 Activities

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ABSTRACT

In 2004, a new project on classical biological control of a hemlock pest, *Fiorinia externa* Ferris (elongate hemlock scale) (Homoptera: Diaspididae), was funded by USDA-Forest Service (FHTET) with work centered at the University of Massachusetts/Amherst. The objectives of the project are to (1) assess current pattern of scale abundance, parasitism, and survival in eastern North America, (2) test the previously proposed hypothesis that host-parasitoid asynchrony accounts for low parasitism and high scale abundance in the northeastern U. S., (3) locate, study, and import new species of parasitoids from Japan and perhaps China as classical biological control agents, and (4) assess host ranges of new parasitoids, followed by their release and an evaluation of their impact on scale density. In 2004, a regional scale survey was conducted, field sites with scale were located, a laboratory scale colony was established, and one trip to Japan made during which scale and parasitoids were found at three locations.

KEYWORDS

Elongate hemlock scale, *Fiorinia externa*, classical biological control, hemlock.

INTRODUCTION

The elongate hemlock scale (EHS), *Fiorinia externa* Ferris, is an armored scale native to Japan that was first recorded in North America on Long Island, New York, in 1908 (Ferris, 1942). Records of EHS exist from Georgia north through Virginia, Maryland, Pennsylvania, New York, New Jersey, Connecticut, Massachusetts, and Rhode Island, and west to Ohio (Scalenet 2005). This scale feeds on a wide range of conifers, including species of hemlock, cedar, fir, pine spruce and yews (McClure 1977 and 1979). Scales are found only on the undersides of needles and crawlers settle principally on the new growth. Scale densities in the United States vary widely, but values from 21 to 164 scales per 100 needles have been reported (McClure and Fergione 1977, McClure 1978). It is possible to find sites with higher densities, but average densities for hemlock stands selected at random have not otherwise been reported. In Japan, densities are much lower: on forest hemlocks, McClure (1986) noted densities as 0.0 to 0.15 scales per 100 needles.

In Japan there is a "landscape" effect on scale density as scales are about 12- to 24-fold more abundant (2.2-3.9/100 needles) on planted hemlocks in landscapes such as temples or parks than in forests (McClure, 1986). In Connecticut, this "landscape" effect either does not exist or is at most much weaker than in Japan, with EHS densities on planted hemlocks being only 1.1- to 4.2-fold higher than on forest hemlocks (McClure and Fergione 1977, McClure 1978). Finally, data from Japan (McClure 1986) suggest that the species identity of hemlock affects scale density very little. Scale density on the eastern North American hemlock *Tsuga canadensis* Carriere, planted in Japan, was 2.9 scales/100 needles, a value that was within the range of scale densities found on native Japanese hemlocks (2.2-3.9) planted in similar landscape settings.

The life-stage phenologies of EHS and its principal parasitoid, *Encarsia citrina* (Crawford), in Connecticut and Japan were determined by McClure (1978 and 1986). In Japan, there is good synchrony between second generation *E. citrina* adults and EHS second instar nymphs (the only stage in which the parasitoid oviposits). In Connecticut, synchrony between these stages was poor. McClure hypothesized that this lack of synchrony in the second generation led to low or fluctuating levels of parasitism in Connecticut instead of the consistently high (>90%) levels seen in Japan. McClure attributed this lack of synchrony to effects of climate, which caused EHS in Connecticut to have less than two full generations per year—in contrast to Japan, where there were two complete generations per year. By extension, this hypothesis predicts better control of EHS south of Connecticut, where two full generations per year occur.

DESCRIPTION OF PLANNED PROJECT

The first goal of this research is to determine if the predicted contrast between the northern and southern parts of the U.S. range of EHS is borne out (i.e., if EHS densities are lower and *E. citrina* parasitism higher in the middle Atlantic states vs. southern New England). The second goal of our research is to study the parasitoids associated with EHS in Japan and determine 1) if the *E. citrina* in Japan is genetically different or not from the population in New England and 2) determine if another parasitoid might be associated with EHS that ovi-

posits in the adult female scale or perhaps in both the second stage female and the adult female (this would be desirable because the adult female is present for much longer and for parasitoids attacking this stage, synchrony issues would be much less critical). In pursuit of such an adult-attacking parasitoid, we plan also to examine populations of *Fiorinia japonica* (Kuwana) in China to see if parasitoids associated with that species might also attack EHS, including adult scales. To meet these broad goals, the following specific objectives were developed for the project:

Obj. 1. Survey EHS density and parasitism from Maryland to Massachusetts.

Obj. 2. Match climates of the northeast U.S. to areas in Japan and China.

Obj. 3. Hire a collector to find *Fiorinia* species on conifers in northern Honshu or Hokkaido.

Obj. 4. Ship dead Japanese parasitoids to the U.S. for preliminary identification; use molecular tools to compare and distinguish between Japanese and U.S. populations of *Encarsia citrina.*

Obj. 5. Hire a collector in China to locate potential *Fiorinia*-collection sites.

Obj. 6. Develop an EHS colony for use in Ansonia FS quarantine lab.

Obj. 7. Collect live parasitoids from Japan and/or China and ship them to quarantine.

Obj. 8. Assess the impact of parasitoids on EHS in Japan and China.

Obj. 9. Start quarantine colonies of all parasitoids recovered.

Obj. 10. Confirm identity of all parasitoids, estimate likely host ranges, submit petitions for releases, and initiate releases.

Obj. 11. Establish parasitoids at field sites in Connecticut and Massachusetts.

Obj. 12. Initiate the field assessment of impacts of introduced parasitoids.

INITIAL RESULTS (FOR 2004)

Work in 2004 addressed Objectives 1-6 only; no work was done on Objective 2. For Objectives 1, 3, 4, 5, and 6, we made the following progress:

Objective 1. Survey EHS density and parasitism from Maryland to Massachusetts. Eight potential EHS survey areas (Figure 1) were identified, four coastal and four inland, stretching from Massachusetts (42 to 42.5 NL) to Maryland-West Virginia (39-39.5 NL). During the survey, hemlock was not found in two of the four coastal zones that we sampled (1: the mid-New Jersey area, and 2: the southern New Jersey-Baltimore, Maryland area). This left six survey regions from which data were collected. These zones were visited in spring of 2004 (April and May) before new hemlock branch growth occurred. Samples for scale density and parasitism were thus based exclusively on needles formed in spring of 2003. Since scales do not fall off needles, a count of scales in early spring of 2004 before new needles were formed provided a summary of the total numbers of scale crawlers that settled in 2003 and how many scales died of parasitism, summed over all scale generations in 2003.

Zones 1 and 2: Massachusetts

Zones 3: Rhode Island and Zone 4: Connecticut and New York

Zone 6: southern Pennsylvania

Zone 8: Maryland and West Virginia

Figure 1. Regions surveyed in spring 2004 to measure *Fiorinia externa* density and parasitism in the eastern United States between Massachusetts and West Virginia.

Within each region, hemlock stands were located with the assistance of local foresters and entomologists, without regard to the status of EHS at a site. In each of the six survey zones, 10 hemlock stands were located and sampled (except one zone with only nine existing stands). Thus a total of 59 hemlock stands were visited and sampled. At each hemlock stand, the surveyor (Suzanne Lyon) first looked at foliage to see if EHS was present. If scale was detected within a 15-minute search, the site was sampled as described here. If no scale was found on any trees at the stand in a 15-minute search period, the stand was classified as having zero scale and samples were not collected; zero-scale stands were included, however, in the data set from which average scale density in the survey as a whole was calculated.

At sites with scale, we sampled five trees, selecting three branches at random from a zone 1-2 m above the ground and spaced around the tree's circumference. From each of the three sample branches, several apical pieces of foliage (each section ca. 20 cm long) were clipped, bagged, and placed in a cooler and returned to the laboratory at the end of the survey. In the laboratory, sets of randomly selected needles (100 per set) were examined, one set from each branch, by selecting ten branch tips (viewed dorsally so that scales were not visible) and taking ten needles from each tip. Sample needles were taken, starting at the collar on the stem marking the division between 2004 and 2003 wood, taking five needles from each side, moving apically until ten needles were obtained. On these needles, all EHS scales (live, dead, parasitized, in any post-egg life stages) were counted. All needles with scale found in the 15 density samples from a site (5 trees x 3 branches per tree) were pooled and held for further use.

To estimate the rate of parasitism and the proportion of living scales in a sample, 150 scales were dissected per hemlock stand. Scales were initially taken at random from the pool of scale-infested needles held over from the density samples. If fewer than 150 scales were present in these samples, all the foliage collected from a site was examined and all scales collected until 150 were obtained or no more were available. All EHS in the per-

centage-parasitism samples were dissected and were classified as parasitized if a parasitoid exit hole was found, if a parasitoid pupa was present, or if upon dissection of the scale body a parasitoid egg or larva was detected.

The number of scales per 100 needles across all 59 hemlock stands sampled was determined by averaging scale numbers over all branches in the study, including three zero-density branches credited for each zero-scale site. The average percentage-parasitism and percentage-live scale (in samples of 150 scales per hemlock stand) over all 59 stands was calculated. All three parameters were then graphed versus collection site latitude (Figure 2).

Scale density per 100 needles was highest in inland Connecticut (at 46.5 scales per 100 needles), dropping to 0.0 scales in samples taken from sites further north (in Massachusetts) and from 0.7 (Pennsylvania) to 5.3 (Maryland + West Virginia) at sites further south. In Connecticut and Massachusetts, scale density was negligible at coastal sites (0 to 0.4). In Pennsylvania and Maryland-West Virginia, hemlocks were not found at coastal sites.

Parasitism rates were generally low, averaging 9.5% across all 59 sites. Parasitism was greatest (14%) at coastal Connecticut sites and lowest at inland Pennsylvania sites (4%). Because no EHS scales were found in samples in Massachusetts, it was not possible to estimate parasitism rates.

The percentage of live scales was greatest in the southern part of the sampled range (42%, Maryland + West Virginia; 38%, Pennsylvania) and decreased to 27-28% in New York-Connecticut-Massachusetts. One site in Connecticut (Tunxis State Forest) sampled on May 29, 2004, had only 4.4% live scale.

Objective 3. Hire collector to find *Fiorinia* species on conifers in northern Honshu or Hokkaido. Naoto Kamata was hired to conduct surveys in Japan for EHS. In August of 2004, assistants provided by Dr. Kamata accompanied Suzanne Lyon, who visited sites in the Nagano Prefecture (Mt. Manza, Mt. Shirane, Kamikochi, the Norikura Kogen Highlands, and Mt. Kiso Koma) to look for hemlocks and EHS. Hemlocks infested with EHS were found at three sites: Kamikochi, the Norikura Kogen Highlands, and Mt. Kiso. Scale density was extremely low at all sites. Counts were not made, but only two to three scales were found per tree when small trees were entirely searched. The most promising site visited was Mt. Kiso. Scales were found on *Tsuga diversifolia* Masters at 1,400-1,700-m elevation and held for rearing. Of the approximately 30 EHS collected, five adult parasitoids were obtained and are being held for taxonomic identification and molecular comparison with parasitoids reared from EHS in Connecticut and Massachusetts. It is very likely that other parasitoids died during rearing if they were younger than the pupal stage when the hemlock needles were picked, which kills the host scale. Sampling in March or November would likely give a better estimate of the true rate of parasitism.

Objective 4. Ship dead Japanese parasitoids to the U.S. for preliminary identification; use molecular tools to compare and distinguish Japanese and U.S. populations of *Encarsia citrina*. Five parasitoid adults were reared from the approximately 30 elongate hemlock scales collected in the August, 2004, trip, suggesting a minimum of 16% parasitism. These parasitoids are currently at the University of Massachusetts-Amherst in 100%

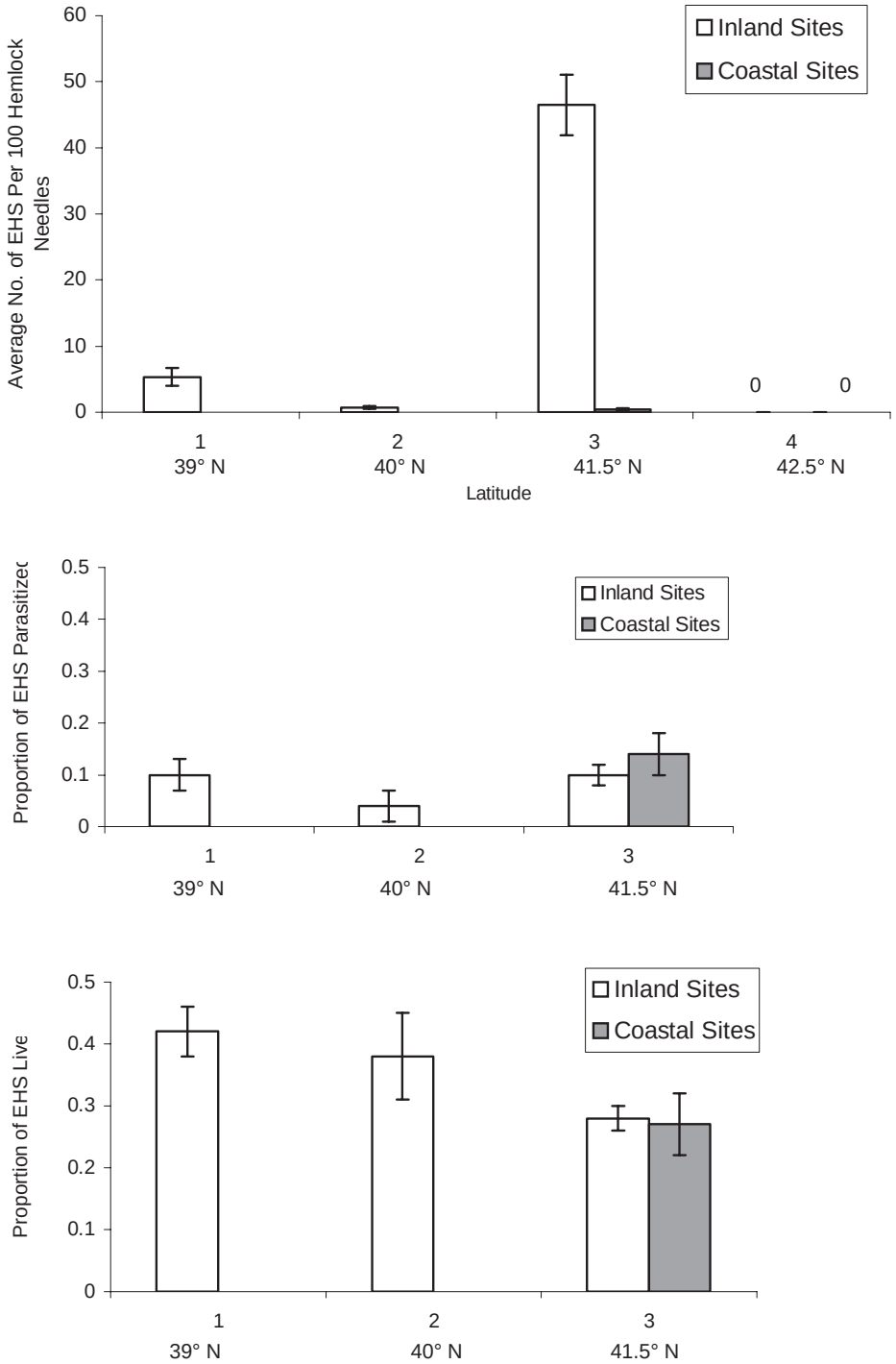

Figure 2. Results of a survey for elongate hemlock scale (*Fiorinia externa*) in the northeastern United States in spring 2004 at four latitudes: Zone 1 (42.5 NL, Massachusetts), Zone 2 (41.5 NL, Rhode Island, Connecticut, and northern Pennsylvania), Zone 3 (40.0 NL, southern Pennsylvania), and Zone 4 (39.0 NL, Maryland and West Virginia). Top: number (mean ± SE) of scales per hundred needles (avg. 15 samples/site and 9 or 10 sites/zone; hemlocks not found in coastal parts of zones 3 and 4; hemlocks present in zone 1 [Massachusetts], but scale was not detected). Middle: proportion (mean ± 95% CI) scale parasitized (of 150 scale, unless scales were limited)—presumably all parasitism was *Encarsia citrina*). Bottom: proportion (mean ± 95% CI) scales on needles still alive.

alcohol and will be used when more specimens are available for identification and molecular comparisons.

Also, in support of eventual importation to quarantine of live parasitoids from Japan, we applied for and received a permit to import live *Fiorinia* spp. scale and its parasitoids from Japan and China into quarantine. This permit is being held by Kathleen Shields at the Ansonia quarantine laboratory and is ready for use when needed.

Objective 5. Hire a collector in China to locate potential *Fiorinia* collection sites. The occurrence of EHS in China is uncertain as the few existing records may be misidentifications. Consequently, Dr. Wu Sanan of the Beijing Forestry University (a scale taxonomist), the collector hired to search for EHS in China, searched in two ways. One search was based on looking for EHS on hemlocks in parks, temples, or other such sites where hemlock are sometimes planted, as scales at such locations are often much more abundant than on the same tree species in natural settings. This approach was carried out in six locations, including Chengdu Botanical garden (Chengdu City, Sichuan Province), the campus of Sichuan University (Chengdu City), the campus of the Sichuan Agricultural University (Ya'an City, Sichuan Province), Qichengshan Forestry Park (Dujiangyan City, Sichuan Province), the Dendrological Garden at the Sichuan Forestry School (Dujiangyan City, Sichuan Province), and the Jiuzaigou Forestry Park (Jiuzaigou County, Sichuan Province). Specimens of *Tsuga* (hemlock) were found only at the last two sites, and no EHS were detected at either site.

The other approach was to find populations of a related scale, *Fiorinia japonica*. Three sites were examined: (1) Beijing, (2) Zhengzhou City (Henan Province), and (3) Yangling City (Shaanxi Province). At the first site, in a July 25, 2004 collection, about 5% of the scale from *Cedrus deodara* (Roxb.) G. Don. had parasitoid emergence holes. At the second site, no evidence of parasitism was found. At the third site (Yangling City), a high density population of *F. japonica* was found on *Picea asperata* Mast. Parasitoids were not reared from the collected scale but a few did have parasitoid emergence holes.

Objective 6. Develop an EHS colony for use in Ansonia FS quarantine lab. To set up a laboratory colony of parasitoid-free EHS on small plants, we tried several approaches in 2004. First we dug small EHS-infested hemlocks (25-50 cm) in Tunxis State Forest in Connecticut, a site that had high densities of EHS. Efforts to transfer crawlers to new, non-infested hemlock seedlings (dug in Westhampton, Massachusetts) were made in three ways. First, we tried tying the foliage of infested seedlings from Connecticut loosely to that of clean seedlings so that crawlers would move over onto the clean trees. Some did, but most settled on their natal branches. In a second attempt, we used EHS-infested fir branches from a Christmas tree plantation in southwest Connecticut. We tried tying these branches very closely to young hemlock. We also tried placing infested needles in paper cones (about 1 inch deep), which were pinned to branches. We assumed crawlers would crawl up the paper cone onto foliage. We thought this would solve the problem of crawlers settling too soon. However, both of these attempts failed because (1) crawlers settling on the hemlock were scarce and (2) mites and thrips from the field material were numerous and the hemlocks were contaminated by these arthropods. A fourth attempt is

currently underway that, while more laborious, is expected to work. Individual female scales with eggs present will be removed from infested foliage and glued onto foliage of fraser fir (about 25 cm tall), which is an excellent host for EHS and shaped to fit well in small cages.

PLANS FOR 2005

Objective 1. Survey EHS density and parasitism in the eastern United States. While our 2004 results partially support the McClure hypothesis (scale density was lower in the southern regions, but parasitism was not higher), the survey needs to be repeated in 2005 at new sites and extended further south. We plan to repeat such a survey in March-May of 2005 covering a region from northern GA to Massachusetts.

Objective 2. Match climates in the northeast U.S. to areas in Japan and China. We need to compare northern Honshu and Hokkaido to Connecticut-Massachusetts to see how important it might be to get parasitoids from Hokkaido. Finding EHS in Hokkaido will require finding it on some non-hemlock host, as hemlocks do not occur on that island. Mike Montgomery and Roy Van Driesche will work together with Kris Abell, the graduate student, to make these comparisons.

Objectives 3 and 4. Collect *Fiorinia* species on conifers in northern Honshu or Hokkaido. Suzanne Lyon and Kris Abell will work with Naoto Kamata to collect more EHS from known sites in Honshu and to find new EHS populations further north. Rearing and dissection of scales from these collections will provide parasitoid adults for taxonomic identification and, through the dissections, information on what host stages each parasitoid species attacks. *Encarsia citrina* specimens will be used for DNA work to determine if they differ from their counterparts already present in the United States. Live specimens of all species will be shipped to the United States to initiate rearing colonies in U.S. quarantine.

Objective 5. Collect parasitoids from other *Fiorinia* species in China. Dr. Wu-Sanan will continue to collect parasitoids from *F. japonica* and determine if these are different species from those found in Japan on *F. externa*. Material from China will be shipped as parasitized scales to the U.S. quarantine laboratory in Connecticut to establish parasitoid colonies for study. Dr. Wu-Sanan will search for *F. externa* on *Abies* or *Cephalotaxus* species.

Objective 6. Develop an EHS colony for use in the Ansonia FS quarantine lab. We will transfer EHS eggs inside female scale covers onto small (20 cm), pesticide-free Fraser fir (greenhouse grown stock from a Connecticut nursery). This species is an excellent host for EHS. These small potted fir trees will be infested by gluing scales with eggs onto fir needles. Scales will be collected by searching foliage of EHS-infested hemlock branches cut from trees at field sites in Connecticut.

DISCUSSION

There are two central questions to answer about a proposed classical biological control program against elongate hemlock scale: (1) Is it possible? and (2) Is it worth doing? We have begun to answer the second question by surveys aimed at establishing the average density of the scale throughout the infested range. In the 2004 survey, elongate hemlock scale was found at high densities primarily in Connecticut. However, heavily infested sites certainly do exist in other states. Dense infestations in the city of Philadelphia in the 1990s were observed by one of the authors (Michael Montgomery), which were believed to have caused a general decline of hemlock in the city. Such high density patches may be of local importance. Further survey work is still needed to determine if the picture that emerged in our 2004 survey is valid. Another concern relative to the importance of this pest is whether it acts synergistically with hemlock woolly adelgid (*Adelges tsugae* Annanad). While it is possible to find these two insects sometimes infesting the same trees, it is not clear at this time if one predisposes the tree to build up in density of the other. Separate Forest Service-funded research on this point is underway by Joe Elkinton of the University of Massachusetts (see Paradis and Elkinton in this volume).

As to the second question of whether or not classical biological control of EHS is feasible, prospects seem good. Many species of armored scales have been successfully controlled by introduction of parasitoids from their native ranges. Data from Japan show EHS there to be at vastly lower density than in Connecticut and show that the change in hemlock species between countries is not sufficient to explain this increase in density. Sheared specimens of the Japanese hemlock *T. diversifolia* in Connecticut have been observed to become heavily infested with elongate hemlock scale (Michael Montgomery, pers. obs.).

Two options are immediately apparent that need to be investigated. First, *E. citrina* in Japan may not be the same species as that name is applied to in the United States. Cryptic species have frequently been discovered in the past when separate populations of seemingly cosmopolitan species of parasitoids are closely compared. Use of molecular tools now makes such comparisons quicker and more robust. However, even if the U. S. and Japanese *E. citrina* populations are found to be the same, another option exists: to import new species of parasitoids. Of greatest interest would be any species able to attack adult female scales, which currently are not attacked in the United States by any parasitoid species. Since this life stage is present for the greatest length of time in the field, a parasitoid attacking it would have few or no problems from a failure of synchrony between parasitoid adults and vulnerable scale stages.

Further work will be required to determine the true importance of this pest and to find the parasitoids associated with it in Japan. However, this effort integrates well with efforts to find additional hemlock woolly adelgid predators in Japan and so the cost of pursuing a project against this pest is somewhat reduced by the overlapping needs of the two projects.

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