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THE USE OF COHORTS TO EVALUATE THE IMPACT OF *ENCARSIA CITRINA* (HYMENOPTERA: APHELINIDAE) ON *FIORINIA EXTERNA* (HEMIPTERA: DIASPIDIDAE) IN THE EASTERN UNITED STATES

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Abstract

Two years of natural enemy exclusion experiments with cohorts of the invasive scale *Fiorinia externa* Ferris were conducted in Connecticut, Pennsylvania, and North Carolina. The parasitoid *Encarsia citrina* Craw had a significant effect on the percentage of *F. externa* that reached reproductive maturity. For cohorts exposed to *E. citrina*, 11% reached maturity in 2006 and 8% in 2007 compared to cohorts protected from *E. citrina*, in which 29% reached maturity in 2006 and 18% in 2007 (averaged across all 3 states). While *E. citrina* exerted some control of *F. externa* density, it was insufficient to maintain *F. externa* density at the study sites at levels comparable to those of the scale in its native range in Japan.

Key Words: natural enemy exclusion, cohorts, parasitoid, scale

Resumen

Se realizaron experimentos de exclusión de enemigos naturales con cohortes de la escama invasora *Fiorinia externa* Ferris por dos años en Connecticut, Pennsylvania y Carolina del Norte. El parasitoide *Encarsia citrina* (Craw) tuvo un efecto significativo en el porcentaje de *F. externa* que alcanzó la madurez reproductiva. Para los cohortes expuestos a *E. citrina*, el 11% alcanzaron la madurez en el año 2006 y el 8% en el 2007 en comparación con los cohortes que fueron protegidos de *E. citrina*, en los cuales el 29% alcanzaron la madurez en el 2006 y el 18% en el 2007 (el promedio de los tres estadios). Mientras que *E. citrina* ejerce cierto control sobre la densidad de *F. externa*, no fue suficiente para mantener la densidad de *F. externa* en los sitios de estudio en niveles comparables a los de la escama en su área de distribución en Japón.

Elongate hemlock scale (*Fiorinia externa* Ferris) (Hemiptera: Diaspididae), indigenous to Japan, was first reported in the United States on Long Island, NY in 1908 (Ferris 1942). Subsequently, elongate hemlock scale spread along the Appalachian Mountains from Massachusetts to northern Georgia, and west to Ohio, Michigan, and Minnesota. During the period from 1998 to 2008, elongate hemlock scale spread further north in New England and increased in density (Preisser et al. 2008a). This northward spread, together with experimental evidence (Preisser et al. 2008b), suggests that elongate hemlock scale is evolving greater cold tolerance and will likely continue to move northward into areas not yet occupied. This change represents a threat to eastern hemlock forests in areas that, up until now, have been protected by cold winter temperatures.

In Connecticut, *F. externa* density varies from 21 to 420 scales per 100 needles (McClure & Fergione 1977; McClure 1978). In its native Japan, elongate hemlock scale densities range from 0.0 to 0.15 scales per 100 needles (McClure 1986). Low densities of elongate hemlock scale in Japan are associated with parasitism by *Encarsia citrina* Craw (Hymen.: Aphelinidae) (McClure 1986). Parasitism rates by this wasp of greater than 90% were reported by McClure (1986) for elongate hemlock scale in Kyoto, Japan, although our surveys found much lower rates (10-40%) (Abell 2010). This aphelinid is a cosmopolitan armored scale parasitoid that is widespread in the United States, attacking many armored scale species (Diaspididae) (Krombein et al. 1979). Its origin is unknown and besides elongate hemlock scale in Japan, there are no documented cases of it independently controlling scale species. Reported parasitism rates of elongate hemlock scale by *E. citrina* in Connecticut range from near zero to greater than 90% (McClure 1981), while in Tennessee, parasitism rates were 20-22% in forested areas and 16-33% in urban areas (Lambdin et al. 2005). Parasitism rates alone, however, do not fully describe the effectiveness of *E. citrina* as a natural enemy of elongate hemlock scale because high rates of parasitism do not necessarily cause population suppression. Because parasitism by *E. citrina* results in low elongate hemlock scale density in its native Japan, but not in the United States, we sought to determine the impact of *E. citrina* on elongate hemlock scale mortality experimentally by conducting a series of parasitoid exclusion trials using artificial cohorts established at field sites in Connecticut, Pennsylvania,

and North Carolina. These sites represent the approximate northern, middle, and southern points of elongate hemlock scale range in the eastern United States. Cohorts were either exposed or protected from *E. citrina*, and scale survival was compared between treatments. The objectives of this study were to determine (1) if parasitism of elongate hemlock scale by *E. citrina* was density dependent under the experimental conditions, (2) if parasitism by *E. citrina* affected mortality rates other than from parasitism per se, such as from host feeding or reduced intraspecific scale competition, and (3) if parasitism by *E. citrina* significantly decreased the percentage of elongate hemlock scale that reach reproductive maturity.

MATERIALS AND METHODS

Site Selection

This study was conducted from Mar to Nov in both 2006 and 2007 at 11 sites in Connecticut, Pennsylvania, or North Carolina. Three sites were in the Bent Creek Experimental Forest in North Carolina (N35 29.694, W82 37.050; N35 29.059, W82 38.338; N35 28.250, W82 38.625); a fourth site, initially selected, was accidentally logged and not replaced. Four sites were in Mont Alto State Forest in Pennsylvania (N39 50.660, W77 30.116; N39 48.782, W77 29.949; N39 47.194, W77 28.350; N39 49.601, W77 27.916), and 4 sites were in the Tunxis or Nathan Hale state forests in Connecticut (N42 00.251, W72 53.702; N42 01.260, W72 59.591; N42 00.096, W72 55.828; N41 45.919, W72 20.572). Hemlock stands in each location used in experiments were selected based on a visual survey of hemlock foliage to confirm the presence of *F. externa*. At each site, 15 hemlock branches were arbitrarily selected, 1 each from 15 different trees located in the stand interior at least 50 m from the forest edge or roads. Selection was done with some bias towards selecting branches with low to moderate *F. externa* density to minimize damage to foliage during removal of scale and to avoid stressed branches that may not have produced adequate new growth.

Cohort Creation

To create study cohorts of elongate hemlock scales, branches were carefully searched and all scales were removed to create scale-free branches that could then be inoculated with *F. externa* crawlers to create a synchronized experimental cohort for the experiment. Branches were inoculated by collecting heavily infested branches from other trees at the same site, inserting these branches into water-pics, and tying them to the cleaned branches to allow movement of crawlers onto the cleaned branch. Branches were moni-

tored weekly for the presence of newly settled crawlers on the cleaned branches and were left unbagged to allow additional immigration of scale crawlers from other local natural sources. As cut branches used for inoculation dried out, they were replaced with fresh branches, and water-pics were refilled on all branches as needed.

When at least 30 settled crawlers were detected on cleaned branches, half of the branches were selected at random and bagged using white polyester fabric with mesh size of 0.1 mm. Before bagging, any scales that had developed to second instars were removed (to prevent introducing potentially parasitized scales into the bags, given that this is the youngest scale instar suitable for *E. citrina* oviposition). However, second instars were rarely present on cohort branches at the start of experiments and the removal of those few second instars that were found did not significantly change average scale densities within treatments.

Data Collection

Branches were left without further manipulations at field sites through the summer and fall until an experiment was terminated. All branches were cut in Nov 2006 or Nov 2007 and shipped to the University of Massachusetts where an inventory of all scales was taken (scale number, life stage, alive or dead, and status as to parasitized or not).

Data Analysis

Unbagged cohorts were analyzed for a significant Spearman's rank correlation between percent parasitism and (1) the number of elongate hemlock scale reaching the susceptible second instar stage, (2) the percentage of mortality to elongate hemlock scales for reasons other than parasitism, and (3) the percentage of elongate hemlock scale that reached maturity. To test for a bag effect, levels of elongate hemlock scale mortality - exclusive of parasitism—were compared for the bagged and unbagged cohorts using a *t*-test. A *t*-test was also used to determine if there was a significant difference in the mean percentage of elongate hemlock scale that reached maturity in bagged versus unbagged cohorts for each state. A *P* value of less than 0.05 was considered significant. All analyses were done using SAS Version 9.1 (SAS, 2003).

RESULTS

Density Dependent Parasitism

In all cases, except North Carolina in 2007, there was no significant correlation between percent parasitism and the density of *F. externa* that

had reached the susceptible stage (second instar) (2006: Connecticut P = 0.050; Pennsylvania P = 0.278; North Carolina P = 0.840; 2007: Connecticut P = 0.745; Pennsylvania P = 0.485; North Carolina P = 0.007) (Fig. 1).

Relationship of Parasitism and Other *F. externa* Mortality

There was a significant negative correlation between percent parasitism and the percentage of scale mortality from sources other than parasitism in Connecticut in 2006 (P = 0.007) (Fig. 2). This suggests that in some cases parasitism by *E. citrina* may decrease intraspecific competition. However, in most cases in this study there was no correlation (positive or negative) between the percentage of *F. externa* mortality from sources other than parasitism and percent

parasitism: Pennsylvania, 2006 (P = 0.823); North Carolina, 2006 (P = 0.537); Connecticut, 2007 (P = 0.839); Pennsylvania, 2007 (P = 0.459); North Carolina, 2007 (P = 0.179) (Fig. 2). The single year × site combination in which this relationship was positive (Connecticut in 2006) had the highest scale density on experimental branches (36.6 scales per 100 needles), compared to combinations where there was not significant correlation: Pennsylvania, 2006 (18.9 scales per 100 needles); North Carolina, 2006 (6.2 scales per 100 needles); Connecticut, 2007 (14.0 scales per 100 needles), Pennsylvania, 2007 (12.8 scales per 100 needles), and North Carolina, 2007 (5.2 scales per 100 needles). Because the mortality rate of first instar scales is high, which might have obscured any potential relationships between parasitism and
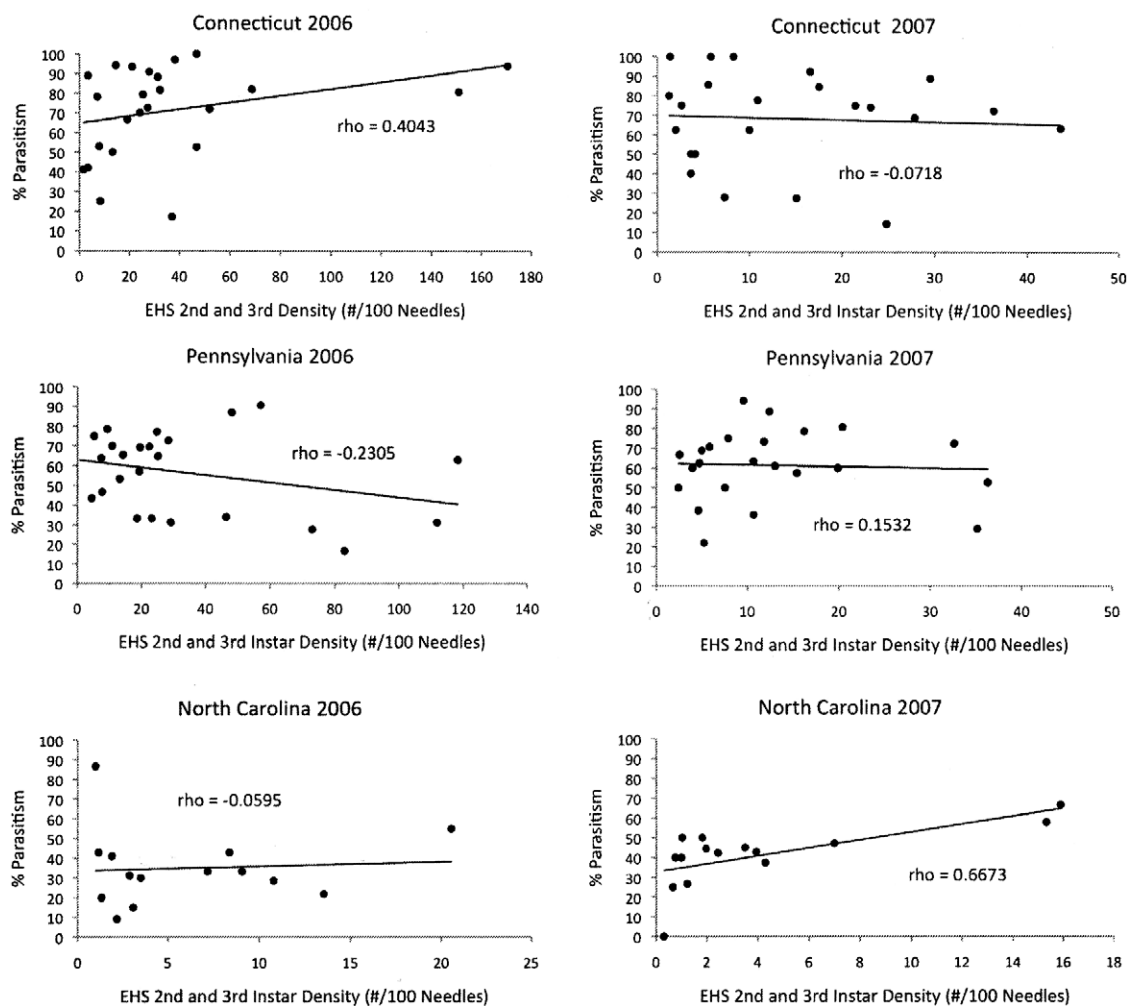

Fig. 1. The relationship between percent parasitism of elongate hemlock scale (EHS) by *E. citirina* and the number of elongate hemlock scale reaching the susceptible stage per 100 needles in unbagged cohorts in Connecticut, Pennsylvania, and North Carolina in 2006 and 2007. Rho is the Spearman rank correlation coefficient.
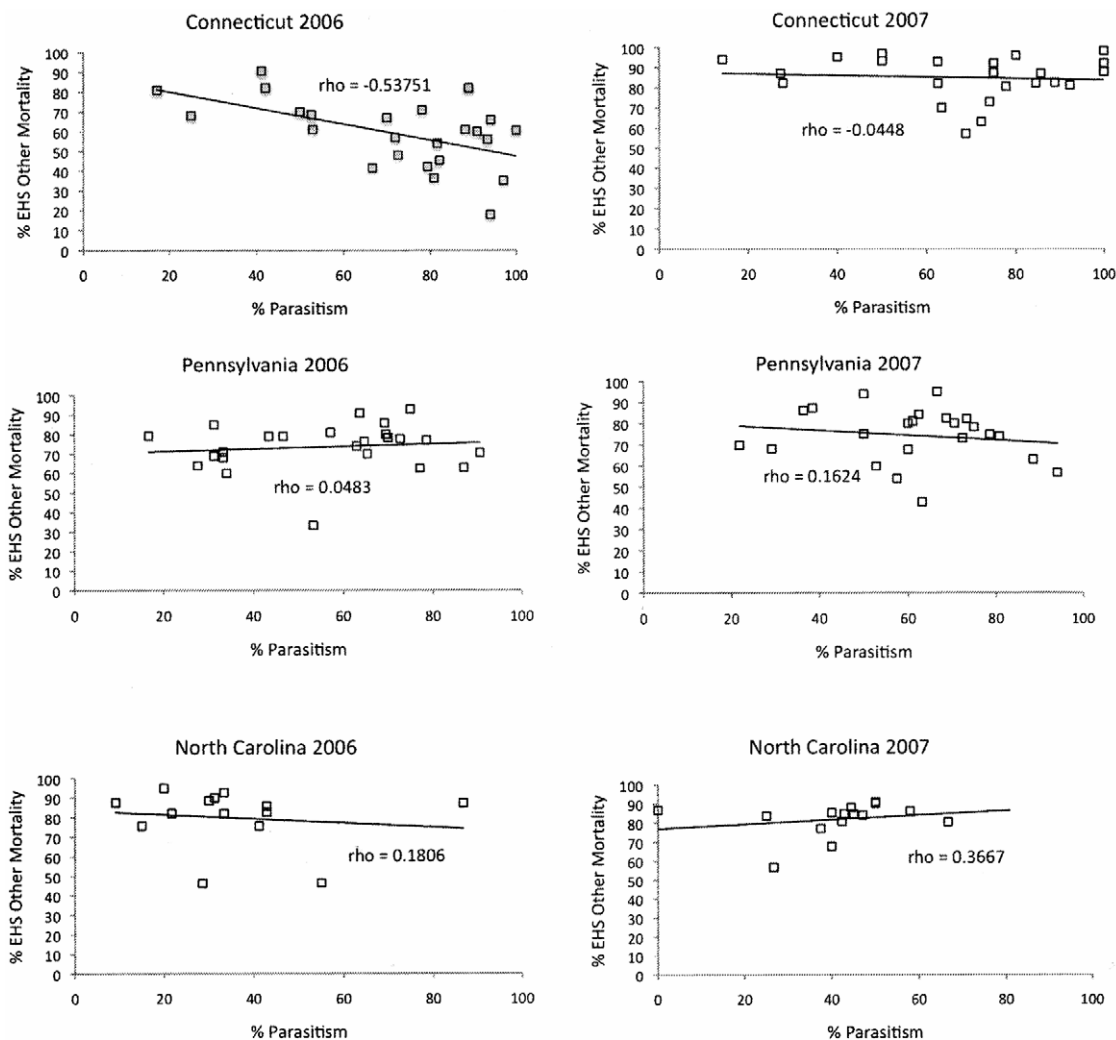
Fig. 2. The relationship between mortality all life stages of elongate hemlock scale (EHS) excluding mortality attributable to parasitism in unbagged cohorts and percent parasitism in Connecticut, Pennsylvania, and North Carolina in 2006 and 2007. Rho is the Spearman rank correlation coefficient.

other mortality, a separate analysis was done with first instars removed. However, the results were the same.

Effect of Parasitism on Percentage of *F. externa* Becoming Adults

There was a significant negative correlation between percent parasitism and the percentage of female elongate hemlock scale reaching maturity (third instar) in Connecticut and Pennsylvania in 2006 and in all 3 states in 2007 (Connecticut: $P < 0.0001$ [2006] and $P < 0.0001$ [2007]; Pennsylvania: $P < 0.0001$[2006] and $P = 0.001$ [2007]; and North Carolina: $P = 0.381$ [2006] and $P = 0.017$ [2007]) (Fig. 3). These results show that parasit-

ism by *E. citrina* reduces the number of reproducing female scales, and, thus, could lower the number entering the next generation.

Comparison of Bagged and Unbagged Cohorts

The percent mortality of elongate hemlock scale, exclusive of parasitism, did not differ significantly between bagged and unbagged cohorts in Connecticut or North Carolina in 2006 or 2007 (Connecticut: $P = 0.785$ [2006] and $P = 0.069$ [2007]; North Carolina: $P = 0.520$ [2006] and $P = 0.660$ [2007]) (Fig. 4a). This result suggests that bagging itself did not affect scale mortality (either positively or negatively), at least in Connecticut and North Carolina, and therefore differences in
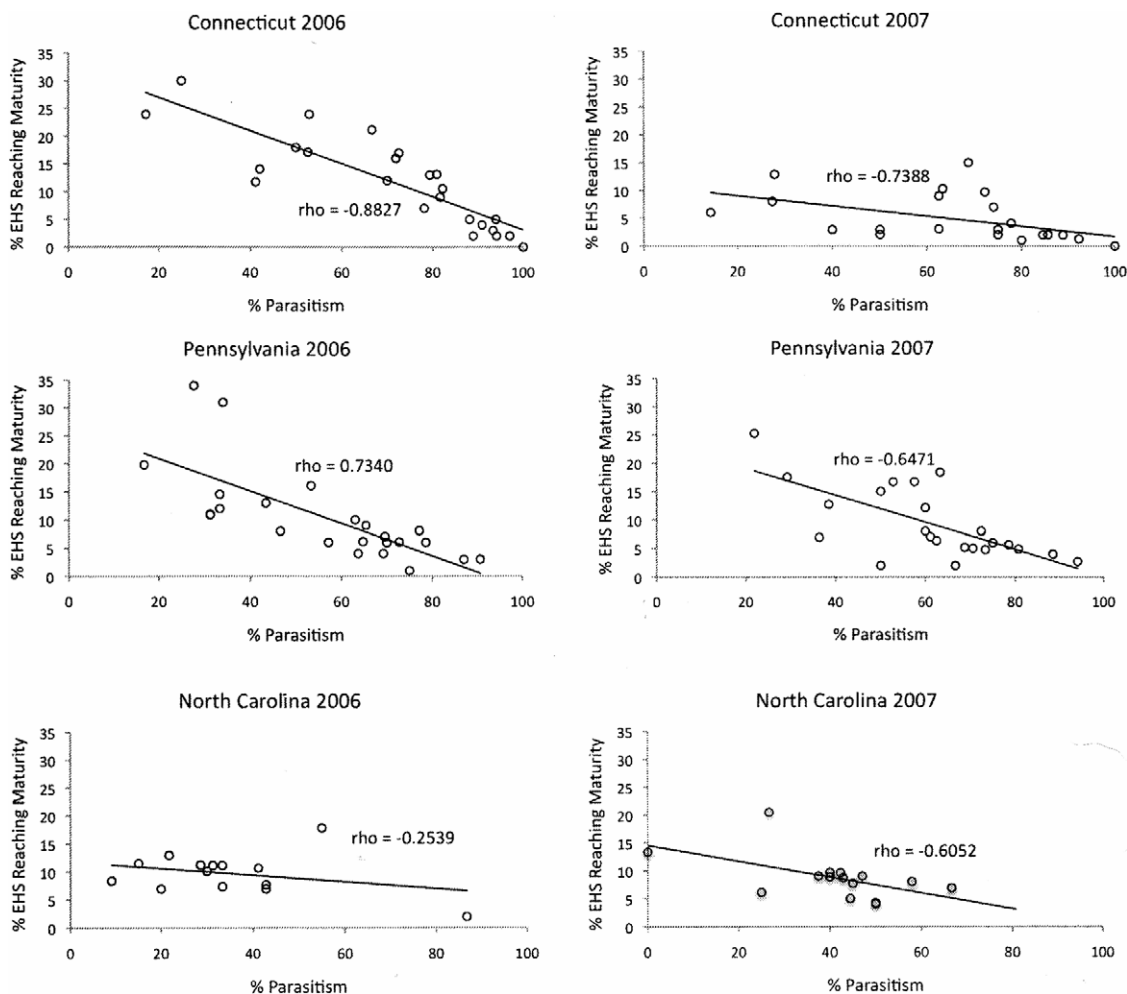
Fig. 3. The relationship between the percentage of elongate hemlock scale individuals (EHS) in unbagged cohorts reaching maturity and percent parasitism in Connecticut, Pennsylvania, and North Carolina in 2006 and 2007. Rho is the Spearman rank correlation coefficient.

total mortality rates between bagged and unbagged cohorts can be interpreted as due to the exclusion of natural enemies. However, a significant difference was seen in Pennsylvania for both years ($P = 0.002$ [2006] and $P = 0.004$ [2007]), suggesting that bagging in that state caused additional mortality.

In 2006, bagged branches in all 3 states had a significantly greater percentage of elongate hemlock scale reaching maturity compared to unbagged branches (Connecticut: $P < 0.0001$; Pennsylvania: $P = 0.023$; North Carolina: $P = 0.030$) (Fig. 4b). In 2007, only in Connecticut did bagged branches have a significantly greater percentage of elongate hemlock scale reaching maturity compared to unbagged branches (Connecticut: $P = 0.0007$; Pennsylvania: $P = 0.4324$; North Carolina: $P = 0.119$). The

lack of significance in Pennsylvania and North Carolina in 2007 was most likely due to several factors: 1) the unintentional exposure of bagged branches to parasitism (Fig. 4c), 2) a significant bag effect on scale mortality in Pennsylvania (Fig. 4a), and 3) the fact that the majority of bagged branches in North Carolina died in 2007, most likely due to a severe drought that occurred in the area. Overall, these results suggest that *E. citrina* exerts substantial control on elongate hemlock scale population growth by decreasing the proportion of the population that reaches the reproductive stage. This conclusion is best supported by the results from Connecticut (because of the lack of confounding factors as did occur in Pennsylvania and North Carolina, as mentioned above). In Connecticut, bagged cohorts had almost 5 times more elon-
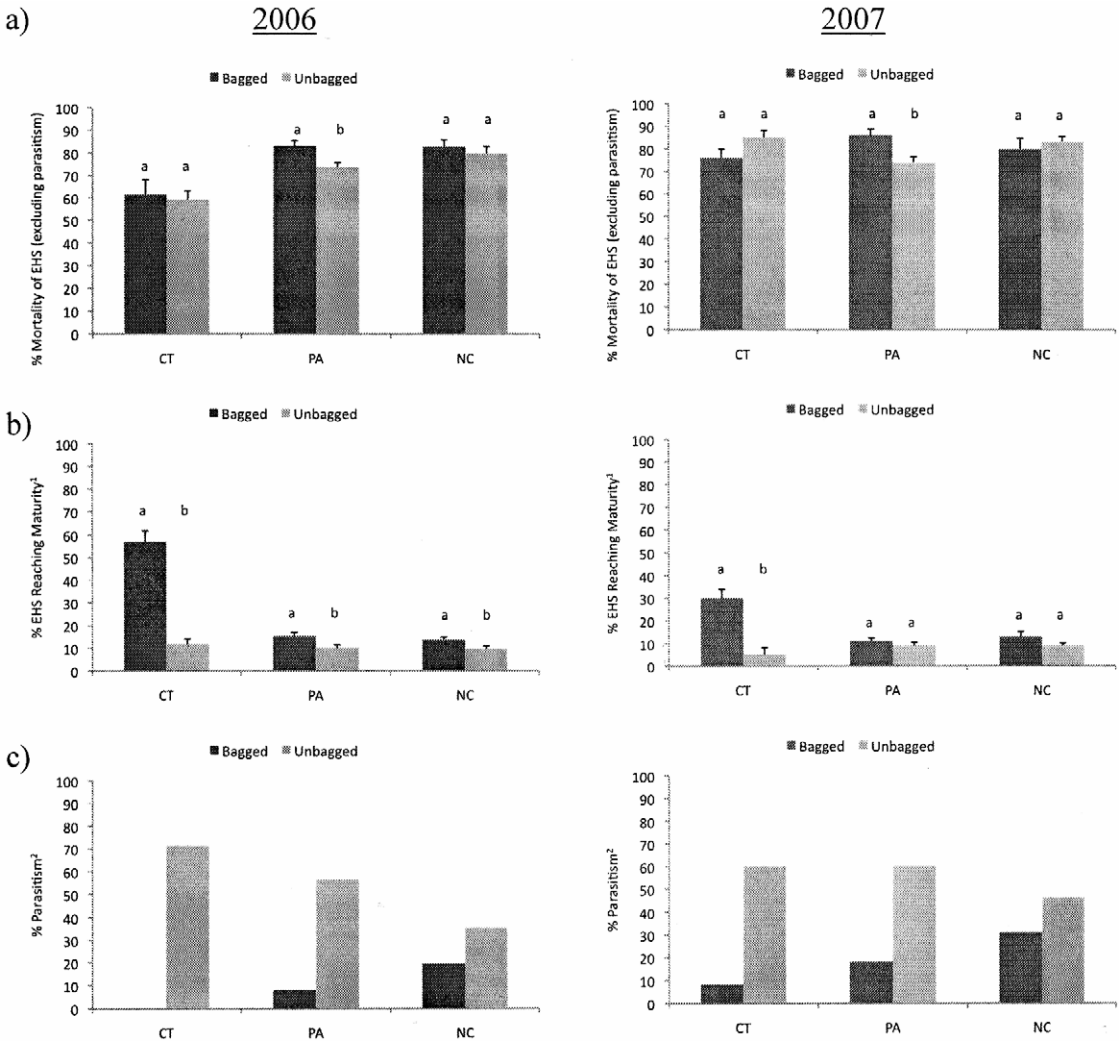
Fig. 4. a) Mean percent mortality (±SE) of all life stages of elongate hemlock scale (EHS) excluding mortality attributable to parasitism in bagged and unbagged cohorts in Connecticut, Pennsylvania, and North Carolina in 2006 and 2007. b) Mean percentage of elongate hemlock scale reaching reproductive maturity (±SE) in bagged and unbagged cohorts in Connecticut, Pennsylvania, and North Carolina in 2006 and 2007. c) Mean percent parasitism of elongate hemlock scale in bagged and unbagged cohorts in Connecticut, Pennsylvania, and North Carolina in 2006 and 2007. Letters above bars (a, b) indicate significant differences ($P < 0.05$) between bagged and unbagged cohorts. [1]Based on the number of individuals of all life stages (first, second, and third instar). [2]Based on the number of individuals entering second instar.

gate hemlock scale reaching maturity than unbagged ones in 2006 and 6 times as many in 2007.

Discussion

Density dependent parasitism of elongate hemlock scale by *E. citrina* was observed by McClure (1977). While we did not find evidence of density dependent parasitism, in our study the density of *F. externa* on manipulated branches (our cohorts) was independent of *F. externa* density on the surrounding branches. Differences in density on experimental branches and local background scale density may have affected the relationship between *F. externa* density and *E. citrina* parasitism rates as seen in this study. However, our results suggest that if parasitism is in fact density dependent as suggested by McClure (1977), then *E. citrina* responds to elongate hemlock scale density at the tree or stand level and not at the branch level, as examined in our study.

Previous studies also suggest that high elongate hemlock scale densities may increase scale mortality due to intraspecific competition (McClure 1979, 1980, 1981). McClure (1979) found that scale densities greater than 100 per 100 needles resulted in a decrease in fecundity in the current generation and density in the subsequent generation. Our results in Connecticut in 2006 suggest that parasitism by *E. citrina* may, in some cases, kill enough scales to lower intraspecific competition, and, thus, lead to a decrease in mortality other than from parasitism. If this relationship exists, then the net effect of parasitism by *E. citrina* is diminished when elongate hemlock scale density reaches levels where mortality from intraspecific competition occurs. This relationship needs further study however, because this pattern was only significant in a single trial. It is likely that in our study, most of our cohort densities were simply not high enough to induce competitive mortality because there were never more than 3 cohorts above 100 scales per 100 needles in each state. Conversely, the fact that we did not find any positive relationship between percent parasitism and other mortality suggests that host feeding or any other source of parasitoid-related host killing other than parasitism was not a an important source of mortality from *E. citrina*.

This study is the first in the United States to evaluate the impact of parasitism by *E. citrina* on elongate hemlock scale by comparing individuals exposed and individuals protected from *E. citrina*. In Japan, cohorts of elongate hemlock scale protected from parasitism were found to increase in density 8.9 to 13.3 times, while those exposed to *E. citrina* increased only 0 to 0.1 times (McClure 1986). Our study focused on the percentage of the cohort that became adult females because this is a direct measure of the impact that parasitism has on a cohort's potential for intergenerational population growth. We found that up to 5 times more females reached maturity in cohorts protected from parasitism compared to those exposed to parasitism. However, given that elongate hemlock scale density is much higher in the United States than in Japan, it would appear that the control exerted by *E. citrina* in the United States, while it decreases the population growth rate, is not sufficient to depress scale density. There are 3 likely explanations for this conclusion: (1) poor host-parasitoid synchrony in the United States, (2) the influence of potentially unknown parasitoids of elongate hemlock scale in Japan, or (3) the possibility that the populations being referred to as *E. citrina* in Japan and the United States are actually cryptic species that differ in their value as natural enemies of elongate hemlock scale. These hypotheses have each been investigated and will be reported in other publications. Regardless of the reason for poor control by *E. citrina*, management of elongate hemlock scale in the United States could potentially benefit from identifying other natural enemies in its native range for importation.

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