The Role of Volatile Terpenoids in the Relationship of the Hemlock Woolly Adelgid and Its Host-Plants

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ABSTRACT

The terpenoid profiles in the needles of the hemlock species were found to be related to geographic distribution of the species and their presumed ancestry. Although a definitive association of individual terpenoids with resistance to the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, was not identified, isobornyl acetate and α-humulene seem to be linked with resistance. Variability in space and time of several components may be as important in determining resistance as the level of a specific terpenoid. Seasonal variability is higher in the leaf cushion, where HWA feeds, than in the needle where the terpenoids are stored. High levels of isobornyl acetate (or its isomer bornyl acetate) are also a characteristic of the foliage of many other North American conifers. We hypothesize that selective pressure from native defoliators such as the hemlock looper, in the absence of native adelgids and scale insects, may have resulted in the phytochemistry of the *Tsuga* species in eastern North America drifting away from protection against sucking insects. Studies of the nutrients and semiochemicals in the leaf cushion xylem ray parenchyma, where the adelgid feeds, are needed to further define the basis of hemlock resistance to HWA.

KEYWORDS

Adelges tsugae, terpenes, chemical ecology, host-plant resistance

PRESENTATION

This talk presented an overview of our three peer-reviewed publications and offered new insight into the previously published data. Over the past several years, we have endeavored to link the volatile semiochemicals of hemlock (*Tsuga*) species from throughout the world to the biological responses of the hemlock woolly adelgid (HWA). We have examined volatile terpenoids in different species (Lagalante and Montgomery 2003), in cultivars of eastern hemlock (Lagalante et al. 2007), and the temporal and spatial variation of terpenoids in eastern hemlock

(Lagalante et al. 2006). We began by developing an analytical method: headspace solid-phase microextraction/gas chromatography/mass spectrometry (SPME/GC/MS). The technique identified 51 semiochemicals (terpenoids) in hemlock needles. Quantitative analyses of the relative abundance of the terpenoids were used to address the following three questions:

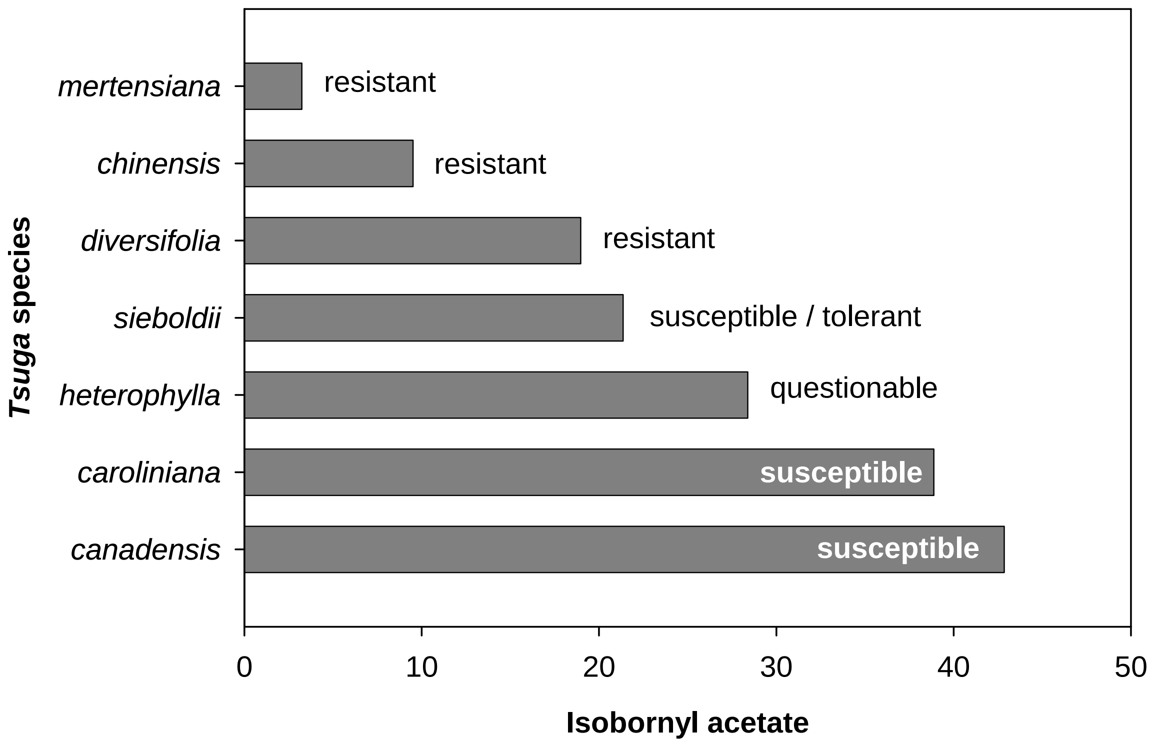
1. How does the relative abundance of terpenoids in each species of hemlock relate to the biogeography and resistance of hemlock species?
2. Are terpenoids different in eastern hemlock selected for horticultural characteristics?
3. How do terpenoids in eastern hemlock vary seasonally in the needles and leaf cushion?

For the first question, the volatile chemical signatures were obtained for seven hemlock species, and principal component analysis (PCA) was used to elucidate similarities and differences in the terpenoids of the species (Lagalante and Montgomery 2003). The PCA extracted two principal components, and the plot of the coefficients corresponded to the geographical location of the species from east to west, except *T. mertensiana*, which is in a separate taxonomic section. (This species behaves as an outlier; hence, all analyses were rerun with it excluded, and the comments below exclude it.) For the first component, which accounted for 75% of the total variance, isobornyl acetate had a much higher loading than any other terpenoid. This terpenoid is most abundant terpenoid in *T. canadensis* and *T. caroliniana* (Figure 1). There are several terpenoids that are lower in the eastern North American species than the other hemlock species: α-humulene, β-caryophyllene, γ-cadinene, δ-cadinene, and γ-muurolene. Of these, α-humulene is most strongly associated with resistance; it is present in 3-4% in the eastern North American species, 6% in *T. sieboldii*, and 11-12% in *T. chinensis*, *T. diversifolia,* and *T. heterophylla* (Figure 1).

A subsequent PCA, using data that were log-transformed to reduce the influence of major components such as isobornyl acetate and α-pinene, grouped *T. caroliniana* closer to the Asian species (see Figure 2 in Lagalante et al. 2007). This arrangement corresponds to the molecular phylogeny of the genus. The separation of *T. canadensis* from the other species appears to be a consequence of high concentrations of piperitone and borneol: both measured approximately 3% in *T. canadensis* and <0.08% in the other species.

The second question was addressed by quantifying volatiles from 13 cultivars of *Tsuga canadensis* using SPME/GC/MS. Multi-dimensional scaling of the dissimilarity of terpenoid profiles for six species and 13 cultivars produces an arrangement (Figure 2) which is similar to that produced by the PCA in Lagalante et al. (2007). Although most of the cultivars of *T. canadensis* have a terpenoid profile that is similar to wild-type *T. canadensis*, four of the cultivars stand apart from the other cultivars: 'Barry's dwarf', 'Callicoon', 'Snowflake', and 'Albospica'. The latter two cultivars belong to the white-tip group; otherwise, the variation in terpenoid chemistry generally did not correspond with the considerable differences in morphological characters observed. 'Albospica' and 'Snowflake' resemble the Asian species in having relatively high levels of α-humulene and germacrene D.

The third question was addressed by measuring the terpenoid content of eastern hemlock in a forest setting over an annual cycle of shoot development. This was related to the temporal feeding pattern of the adelgid that has two generations on hemlock each year. The



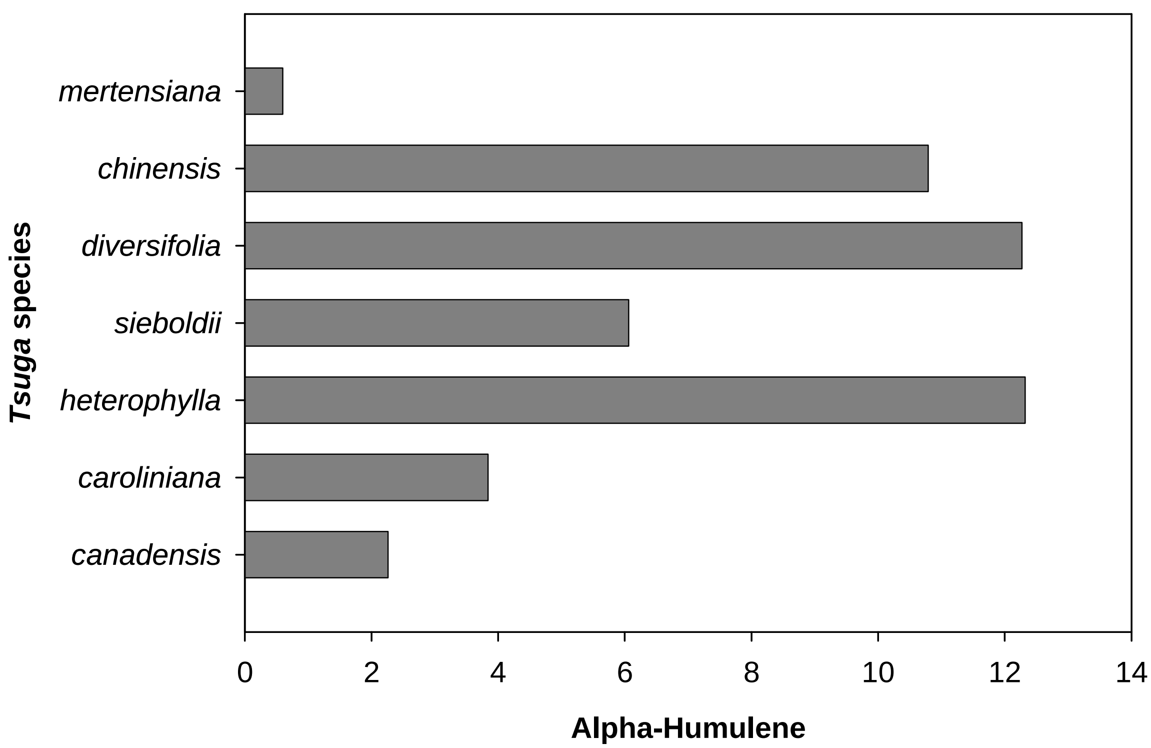


Figure 1. Relative percentage for two of the 51 terpenoids recovered from needles of seven species of hemlock. The resistance of each species to *Adelges tsugae* in the eastern United States is based on Del Tredici and Kitajima (2004) and the authors' unpublished evaluations.

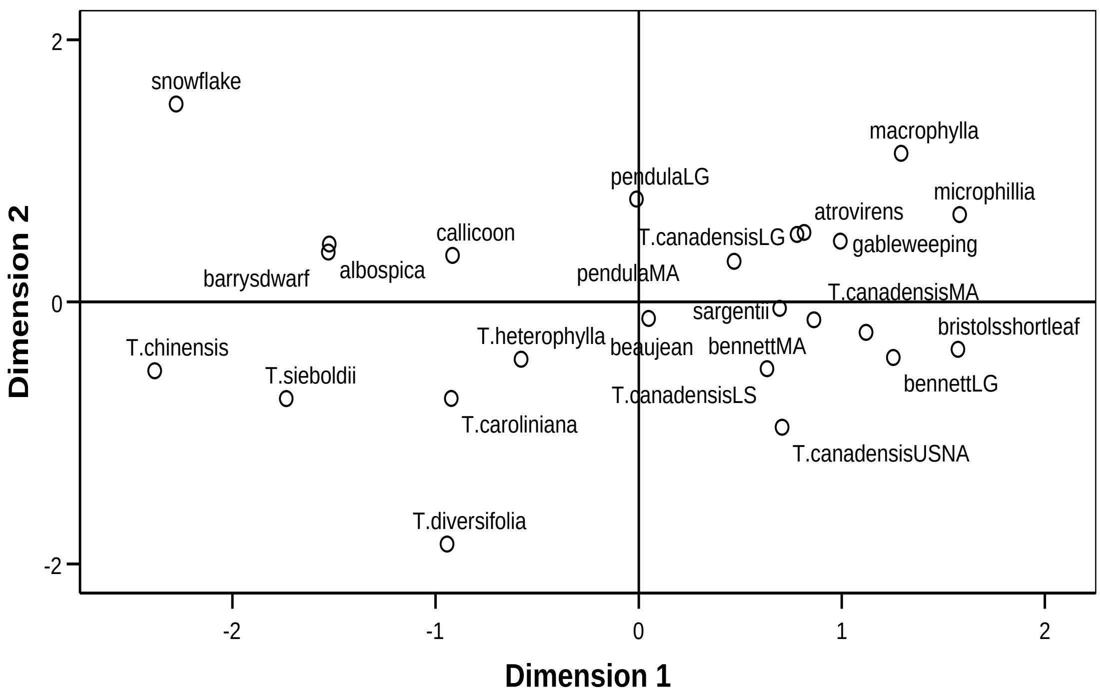


Figure 2. Derived configuration based on multidimensional scaling using Euclidian distances calculated for 23 terpenoids in six hemlock species and 13 named cultivars of *T. canadensis* (lower case names). The non-cultivar *T. canadensis* are from four locations (LG, MA, USNA, and LS) and the cultivars 'Bennett' and 'Pendula' were replicated in the two arboreta (LG and MA).

spring (progrediens) generation is shorter, lasting from May to July, with the adelgid feeding on the previous year's growth. This is also the same part of the hemlock twig where its mother fed. The following generation (sistens) appears in July and settles on the new growth, but it enters diapause and does not begin growing until the fall. The adelgid develops during winter when temperatures are above freezing, becoming an egg-laying adult in late winter or early spring. In additional to seasonal changes, this study took a closer look at the micro-site where the adelgid feeds. Although the terpenoids are stored in the resin canal located in the hemlock needle, the adelgid feeds in xylem ray parenchyma in the leaf cushion. To examine the variation of terpenoid composition in space and time, the needle and leaf cushion of the current and previous years' growth were analyzed over a one-year period. Comparison of these four fractions found significant variation for all of the 23 terpenoids present above 0.1%, including complex interactions between the temporal or spatial factors. Isobornyl acetate and α-humulene increased in the leaf cushion as it matured, whereas α-pinene, myrcene, and germacrene D decreased. Ordination and factor analysis revealed that the terpenoids vary more over time in the leaf cushions than in the needles, with the overall terpenoid composition in the leaf cushion becoming more similar to that in the needle as the tissues mature. The influence of age of the leaf cushion on terpenoid levels was greatest for myrcene and germacrene D, which measured >16% in immature leaf cushions and <4% in mature leaf cushions and immature and mature needles (Figure 3). By entering a non-feeding diapause during the late spring and summer, the sistens HWA would avoid the unstable, variable levels of terpenoids in the immature leaf cushion.

Alpha-humulene, which was associated with resistance in the study comparing species, was relatively more abundant in the leaf cushion than in the needle, especially during the periods when the adelgid is feeding (see Figure 3). Higher levels at the specific feeding site during the actual time period of feeding is what one would expect in a resistant host if the terpenoid were toxic. However, *T. canadensis* is susceptible. An explanation may be that the total terpenoid level in the leaf cushion is only about one-tenth the level in the needle; thus, the absolute level of α-humulene in the leaf cushion may not be toxic. It should also be noted that all of these studies were made using lightly infested or uninfested, healthy hemlocks in order to avoid herbivore induced effects.

In order to determine the extent that terpenoids are a basis of resistance, we, like the adelgid, must probe the leaf cushion. The species comparison, which looked at terpenoids in the needles, needs to redone with the leaf cushion. A key to understanding hemlock resistance to HWA is in-depth knowledge of both the primary and secondary chemistry of the xylem ray parenchyma cells on which the adelgid feeds. These cells store starches and lipophilic compounds, including steryl esters and waxes. Thus, the parenchyma cells may not only provide the adelgid with nutrients but also the precursors of the woolly wax it uses as a protective covering. The parenchyma cells are produced by the cambium, and the radial parenchyma is a living link from the bark to the inner wood. Since disturbance of this link could lead to whole-tree trauma, the *de novo* synthesis of terpenoids in response to feeding stress may be more critical than static reserves of terpenoids. Thus, an examination of the induced changes in the chemistry of the ray parenchyma is also critical to understanding the basis of hemlock resistance to HWA.

The major conclusions from these works can be summarized as follows:

- Terpenoid profiles in hemlock species relate strongly to geographic distribution and phylogeny.
- By feeding in mature leaf cushion, HWA avoids the high variability of terpenoids found in the developing needles.
- High levels of isobornyl acetate in the two eastern North American species of hemlock may reflect their co-evolution in the presence of defoliators and the absence of sucking insects.

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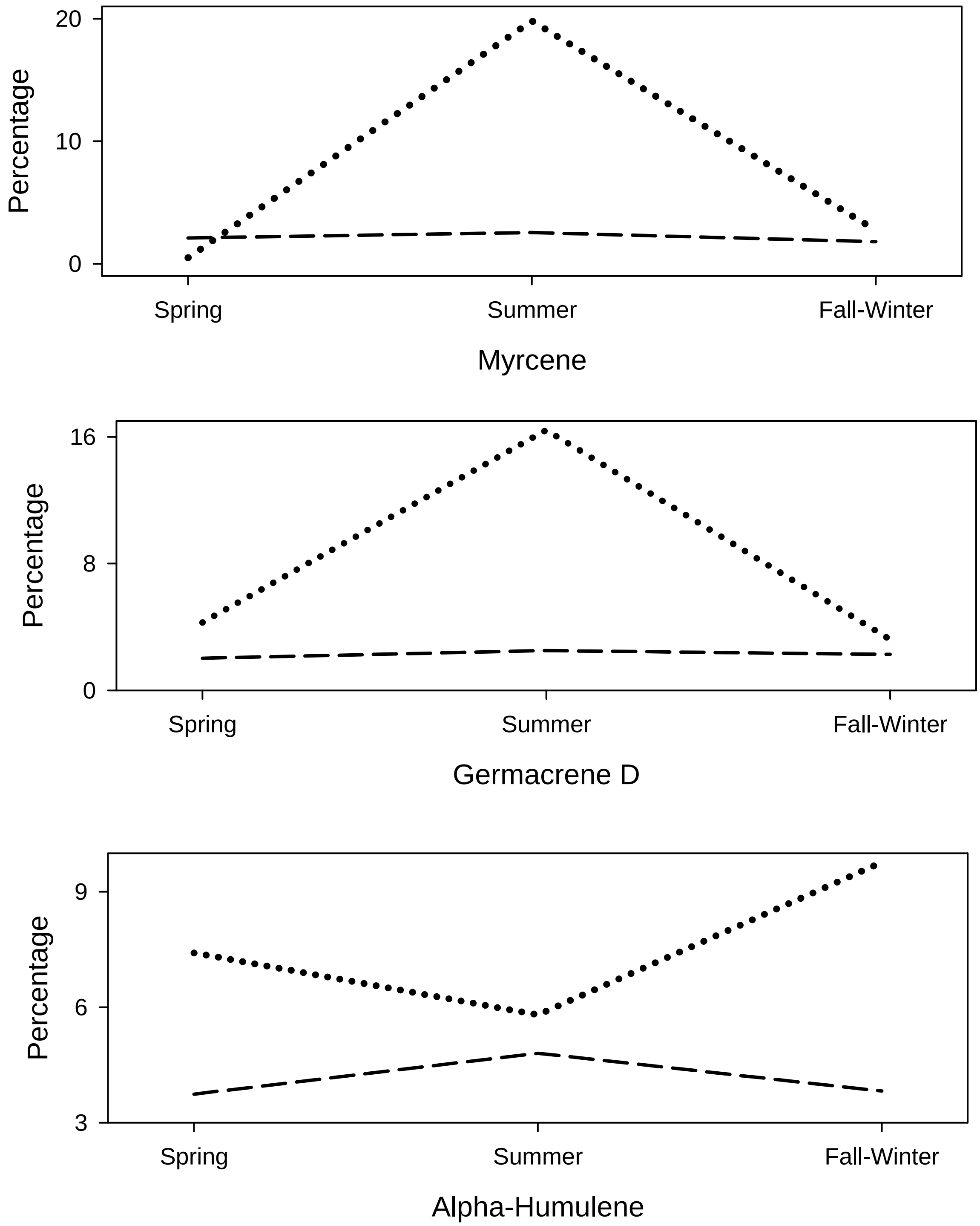

Figure 3. Seasonal comparison of selected terpenoids as the percentage of total terpenoids in needles (dashed line) and leaf cushions (dotted line). Spring is measured on the previous year's growth (penultimate internode) on which HWA both settles and feeds; Summer is the new growth (terminal internode) on which HWA settles and enters diapause, and Fall-Winter is the now-mature terminal internode on which HWA is now feeding.

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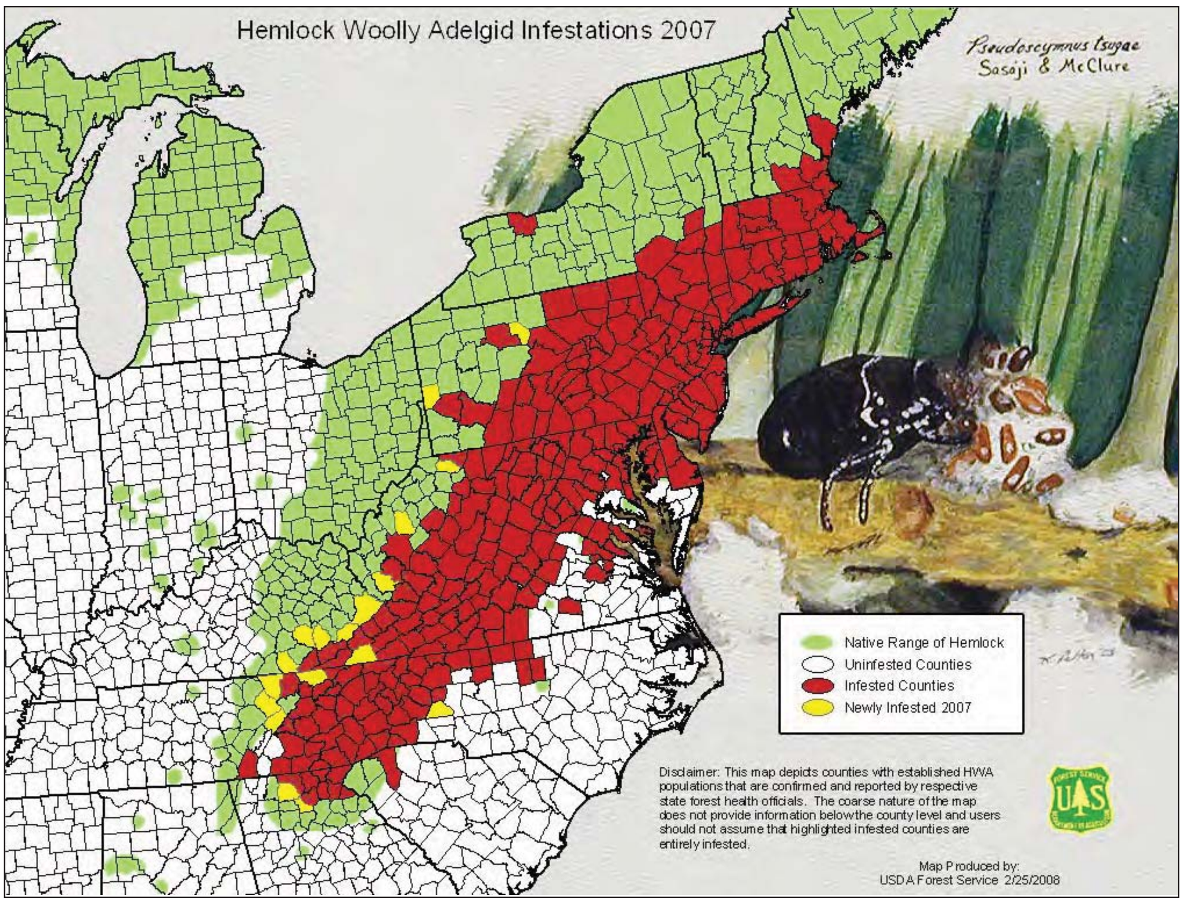

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