Assessment of Environmental Conditions and Preferences for Rearing the Balsam Woolly Adelgid (Hemiptera: Adelgidae) on Host Material[1]

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J. Entomol. Sci. 50(3): 157–167 (July 2015)

Abstract The optimal environmental conditions for rearing the balsam woolly adelgid, *Adelges piceae* (Ratzeburg) (Hemiptera: Adelgidae), on its natural host, Fraser fir, *Abies fraseri* (Pursh) Poir, was determined by varying temperature, photoperiod, and humidity as well as by evaluating texture and geotaxic preferences. Five locations with a set temperature and relative humidity (RH) were chosen to test for optimal environmental conditions: three environmental chambers maintained at 17, 20, and 25°C with 75% RH; an insectary maintained at 21.1°C and 72% RH; and a greenhouse with varying temperature and RH levels. Overall, 20°C appeared to be the optimal temperature for development, and 25°C was the least favorable of the temperatures tested. Comparing Fraser fir cuttings to seedlings, seedlings (15.51/cm) were five times more infested than cuttings (3.02/cm), and the percentage of adult survival was higher on seedlings (67%) than on cuttings (17%). The highest percentage of adelgids reaching full development occurred in adelgids that inserted around the buds. Although the numbers were very low, full development was reached on logs in all five environments. Texture of the bark plays a role in *A. piceae* preference, with twice the number of crawlers inserting into medium-textured bark compared to rough-textured bark. *Adelges piceae* showed positive geotaxis preference, with the most favorable insertion sites on the bottom sides of vertical bark discs. Future rearing of adelgids in the laboratory should be on logs or seedlings in an area of sufficient size to permit adequate airflow at 20°C and 75% RH.

Key Words *Adelges piceae*, *Abies fraseri*, insect rearing, host material

Since its introduction into the United States, the balsam woolly adelgid, *Adelges piceae* (Ratzeburg) (Hemiptera: Adelgidae), has spread through the Southern Appalachian Mountains, killing thousands of hectares of Fraser fir, *Abies fraseri* (Pursh) Poir, an important natural resource and prominent Christmas tree species. Fraser fir occurs naturally only in the Southern Appalachians and is the dominant tree at the highest elevations (Dull et al. 1987). Fraser fir is a subalpine species that can live for 150 yr. It is not only important for its scenic beauty but also for providing a home for many wildlife species, such as the Carolina northern flying squirrel,

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Glaucomys sabrinus Shaw, and the spider, *Microhexura montivaga* Crosby & Bishop (Araneae: Dipluridae) (Cooper et al. 1977, Terwilliger and Tate 1994).

Fraser fir serves as one of the most popular Christmas tree species in North America, and the Christmas tree industry provides an important economic resource for mountain communities. Virtually all Fraser fir Christmas trees require treatment for *A. piceae* one or more times during a 5–10-yr rotation, and the threshold for treatment is a single infested tree. Chemical insecticides are currently the only effective means for controlling *A. piceae*, costing the Christmas tree industry more than US$1.5 million/yr (Potter et al. 2005).

The availability of laboratory-reared *A. piceae* year-round would be useful for research on host plant resistance, biological control (rearing predators), and other approaches to control this insidious pest. In the rearing process, insects are removed from their natural setting and have stresses inflicted upon them that they never face in nature (Cohen 2003). One can try to mitigate these issues by presenting the insect with optimal conditions, such as temperature, humidity, geo- and phototaxic preferences, and nutrition (Bell et al. 1981). However, research on optimal conditions and rearing for *A. piceae* is lacking.

Our first objective was to determine the optimal environmental conditions for rearing *A. piceae* on natural host material, including Fraser fir logs, excised branch cuttings, or seedlings, by varying temperature, photoperiod, and humidity. Amman (1968) found that any temperature between 11 and 20°C coupled with any level of relative humidity (RH) between 75 and 98% provides for relatively equal hatching success for *A. piceae*. Atkins and Hall (1969) found that vigor of first-instar larvae (crawlers) dropping to the forest floor declines as temperatures increase, and warm periods may reduce crawler populations considerably. Therefore, we hypothesized that rearing success would be equally successful in chambers maintained at 17 or 20°C with RH ranging between 75 and 98%, whereas *A. piceae* development at 25°C would result in the production of the lowest numbers of crawlers among all treatments.

Our second objective was to evaluate noninfested Fraser fir bark discs to further determine texture and geotaxic preference as well as the percentage of crawlers that actually insert into their host material. Several studies have shown that orientation of crawlers is predominantly photonegative, although some crawlers orient toward the light (Balch 1952, Atkins and Hall 1969, Havill and Foottit 2007).

Materials and Methods

To address our first objective, we chose five locations with various combinations of temperature and RH to test for optimal environmental conditions: three environmental chambers maintained at either 17, 20, or 25°C and 75% RH; an insectary chamber maintained at 21.1°C and 72% RH; and a greenhouse with varying temperature and RH. The mean temperature and RH were recorded for each environment and were 17.4°C (51.2% RH) in the 17°C chamber, 20.1°C (65.4% RH) in the 20°C chamber, 26.2°C (80.6% RH) in the 25°C chamber, 21.3°C (65.2% RH) in the insectary, and 24.2°C (56.1% RH) in the greenhouse.

Thirty-seven Fraser fir seedlings had been stored in the greenhouse before this study. One month before starting the study (February 2012), seven seedlings were left in the greenhouse and 30 seedlings were distributed throughout the other environments (4 in each chamber, 18 in the insectary) to allow for acclimation to each environment. All seedlings were 4–5 yr old and were potted in 25.4-cm-tall containers. Infested and noninfested Fraser fir logs were collected from trees in an abandoned Christmas tree farm in Avery Co., NC, in March 2012 and returned to North Carolina State University campus in Raleigh. The infested Fraser fir logs were set up using the suspended bolt method that mimics natural infestation and is an effective technique for artificially infesting fir trees with *A. piceae* (Newton et al. 2011). Infested and noninfested logs had a moistened shop towel (industrial strength absorbent paper towel) wrapped around each end of the log to slow drying.

Cuttings in each environment consisted of one excised branch from each of the five noninfested trees that were collected, allowing comparison among the same five genotypes within each environment, totaling 45 cuttings (5 in each 17 and 25°C chamber and greenhouse, 10 in the 20°C chamber, and 20 in the insectary). To determine whether cuttings could be used as surrogates for whole trees, 12 excised cuttings corresponding to each of the 12 seedlings in the chambers (four cuttings per incubator) were taken to see whether there was a significant difference in total *A. piceae* per centimeter found in a cutting compared to the seedling source (Newton et al. 2011). The cuttings were placed into sand-filled 50-ml plastic centrifuge tubes, randomized in trays, and placed into plastic tubs. To keep the cuttings hydrated, the tubes and trays had small holes cut in the bottoms, and water was added to saturate the sand. Seedlings, cuttings, and logs in all five environments were watered twice a week, and temperature, RH, and the condition of each were recorded.

Each incubator contained one infested log (61 cm in length) suspended horizontally over one perpendicular noninfested log (30.5 cm in length), the Fraser fir seedlings with their corresponding excised cuttings propped up in each seedling's container, and the cuttings from the five noninfested trees. Saturated sodium chloride was placed in trays in the bottom of each incubator to maintain RH (Wexler and Hasegawa 1954). Based on the observation of Amman (1968) that crawlers on logs in direct sun move directly into shaded crevices or to the underside of the log, a fluorescent grow light with a timer installed to provide light for the cuttings (16:8 light:dark photoperiod) was placed directly above the suspended infested log to force the crawlers toward the underside and to drop onto the noninfested material below.

In the insectary, four racks were set up with two infested logs (122 cm in length) suspended from each rack. Above the racks were fluorescent grow lights with a timer installed to provide light for the cuttings (14:10 light:dark photoperiod). Below the infested logs were two noninfested logs (61 cm in length), with one log perpendicular and one log vertical; four Fraser fir seedlings; and five excised cuttings. Two seedlings were added to a side bench as a control. In the greenhouse, two infested logs (122 cm in length) were suspended above three noninfested logs (61 cm in length), with two logs perpendicular and one log vertical; seven Fraser fir seedlings; and cuttings from the five noninfested trees.

The infestation level was determined for *A. piceae* development 13 weeks after suspending logs in the chambers and the insectary and 11 weeks after suspending logs in the greenhouse. Infestation level was determined using a dissecting microscope to examine all sides of the cuttings and to count the number of crawlers, settled nymphs, adults, and eggs present. The location (top, middle, bottom, needle, needle cushion, branch, bud, and crevice) of the settled crawlers was also noted. To determine the infestation level in seedlings, a subsample was obtained from each seedling, based on a scheme developed by Newton et al. (2011).

On each side of the log, three spots were chosen (top, middle, and bottom), with a diameter of 4.5 cm and total area of 15.90 cm^2/spot, to create 12 spots in total and a total area of 190.8 cm^2 for each log observed. The numbers of crawlers, settled nymphs, adults, and eggs present were recorded at each spot. If there were branches on the log that had visible woolly masses, it was noted or recorded if located at an observed spot, but most of the logs did not have branches.

To address objective 2, noninfested Fraser fir bark discs were cut with a cork corer (3 cm in diameter), and under a microscope (20×), texture was determined: rough, a lot of texture or "roughness;" medium, roughness broken up with flat or smooth areas; and smooth, smooth and completely flat. In total, 15 discs was set up in two chambers, with four vertical discs (leaned up against the side wall of the Petri dish), four horizontal discs in the 17°C chamber, and three vertical discs and four horizontal discs in the 20°C chamber. Petri dishes were filled with sand with a piece of shop towel over the sand (kept moist to keep the bark discs hydrated), and bark discs were placed on top. Crawlers and eggs were placed by hand in the center of each disc and were monitored daily. Observations included the number of hatched and unhatched eggs, stationary or mobile crawlers, and their location on the wood discs (e.g., bottom left in a crevice). Change in appearance was also noted once the crawlers inserted and started to feed (e.g., presence of wax, change in color). Once there were no additional crawlers, the eggs that remained were unhatched and appeared damaged, or both, the study was ended, and the final number and location of crawlers that inserted into the bark was recorded.

Statistical analyses. Analyses of variance (ANOVAs) for cuttings and seedlings were performed, and pairwise comparisons were analyzed by the Tukey–Kramer honestly significant difference test (SAS Institute 2010). Transformations were applied to bring the distributions closer to normality and to make the variances more homogeneous. The ANOVA for logs was not carried out because of low infestation. Objective 1 incorporated the following effects: environment, sample × environment, sample type (cuttings, seedlings, logs, and paired samples), and position (bud, stem, needle cushion, and needle). Objective 2 incorporated the following effects: topography, environment, position (horizontal or vertical), and environment × position. Data for both studies are presented as least squares means, and details specific to each study are provided in the Results and Discussion.

Results and Discussion

For determining optimal environmental conditions, the ANOVAs for cuttings and seedlings were performed with log transformations to account for scale effects. There was a significant environmental effect ($F = 5.48$, df = 4; $P = 0.0451$) as well as a significant interaction between seedling source and environment for cuttings ($F = 0.84$; df = 16; $P = 0.6446$). The infestation of all *A. piceae* per centimeter length was highest in the insectary and lowest in the 25°C chamber (Table 1). Approximately 17% of the settled crawlers survived to the adult stage with complete development

Table 1. Mean (±SE) number of adelgid life stages per centimeter of Fraser fir cuttings in response to different environmental treatments.

Environment	All Stages*	Stationary Crawlers	2nd Instars	3rd Instars	Adults	Eggs	% Survival**
Insectary	4.20 (2.77) a	0.93 (0.14)	2.64 (1.04)	0.07 (0.04)	0.42 (0.42)	0.13 (0.13)	45
Greenhouse	3.04 (2.77) ab	0.58 (0.5)	2.47 (2.41)	0	0	0	0
20°C	2.89 (1.96) ab	0.76 (0.1)	5.02 (3.59)	0	0	0	0
17°C	0.16 (2.77) ab	0.02 (0.02)	0.13 (0.04)	0	0	0	0
25°C	0.07 (2.77) b	0.07 (0.1)	0	0	0	0	0
All		0.47 (0.12)	2.05 (0.9)	0.01 (0.01)	0.08 (0.08)	0.03 (0.03)	17

* Means within a column followed by the same lowercase letter are not significantly different ($\alpha = 0.05$; Tukey's honestly significant difference test).
** Percentage of survival from stationary crawler to adult stage.

(0.42/cm) and egg production (0.13/cm) in the insectary. All of the cuttings died before the experiment ended, with the shortest longevity in the greenhouse (44 d) and longest in the insectary (73 d).

No significant differences were found among seedlings ($F = 2.20$; df = 4; $P = 0.0731$). The infestation of all *A. piceae* per centimeter length was highest in the 20°C chamber and lowest in the 25°C chamber (Table 2). Full development was seen in the insectary (0.13/cm) and greenhouse (0.09/cm). Eggs were observed in the 17°C (0.05/cm) and 20°C (0.04/cm) treatments, but no adults were observed. The percentage of survival appeared to be much higher on seedlings (67%) than cuttings (17%). Some seedlings remained alive at the end of the study, with the shortest longevity in the three chambers (76 d) and longest longevity in the insectary (111 d). Between cuttings and seedlings, *A. piceae* preference for insertion into the buds was consistent (Fig. 1), which was also the optimal position for *A. piceae* to reach full development (Fig. 2), perhaps because of greater nutrient content.

There was a significant environmental effect ($F = 18.79$; df = 2; $P = 0.0006$) as well as a significant effect between the 12 paired cuttings and seedlings ($F = 9.63$; df = 1; $P = 0.0126$) in the chambers, suggesting that paired samples of cuttings are not indicative of equal success as seedlings because cutting longevity was compromised. The infestation of all *A. piceae* life stages across all environments among paired cuttings and seedlings was highest in the 17°C chamber (14.55/cm) and lowest in the 25°C chamber (0.27/cm), and seedlings (15.51/cm) were five times more infested with *A. piceae* than cuttings (3.02/cm) (Table 3).

Overall, seedlings lived longer than cuttings across all environments (26.1 ± 11.8% versus 100 ± 0.00%, respectively). Cutting and seedling death is possibly due to the high infestation level on a small surface area where *A. piceae* competed for nutrients. Once a critical level of nutrients was consumed by the adelgids, further development was inhibited and resulted in death of the cuttings and seedlings. Furthermore, due to their lack of roots, excised branches are under more physiological stress than seedlings, which alters normal active growth (Newton et al. 2011). In terms of future research involving host resistance or rearing in the laboratory, excised branches might not be adequate substitutes for whole trees.

In every environment, *A. piceae* on logs developed into adults, but no eggs were observed (Table 4). Because woolly masses are easy to observe, looking outside of the selected sample location showed there were other adelgids, but the overall infestation levels were very low. This is possibly because the length of the study did not provide sufficient time to reach full development or that development was slowed due to log stress (e.g., the logs were cut and dried out and were no longer providing suitable metabolites for *A. piceae* needs and therefore did not appear favorable to *A. piceae* once they landed on them). The sampling technique could have also been inadequate in that there may be more hiding places, making the adelgids harder to see; the spots observed could have been less infested by chance; or the size of the logs compared to the seedlings and cuttings requires more time to reach a high infestation rate. Future studies should be planned at longer time intervals to allow the logs more time to become infested.

The texture and geotaxic study showed an interaction between environment and position. Vertical discs in the 17°C chamber had the highest percentage of inserted crawlers (17.3%) among all temperature–disc position combinations (Table 5).

Table 2. Mean (±SE) number of adelgid life stages per centimeter of Fraser fir seedlings in response to different environmental conditions.

Environment	All Stages*	Stationary Crawlers	2nd Instars	3rd Instars	Adults	Eggs	% Survival**
Insectary	1.26 (0.22) a	0	4.82 (1.04)	0.10 (0.08)	0.13 (0.09)	0	0
Greenhouse	1.58 (0.35) a	0	6.22 (1.8)	0	0.09 (0.04)	0	0
17°C	0.22 (0.75) a	0.19 (0.07)	0.04 (0.04)	0.05 (0.05)	0	0.05 (0.04)	0
20°C	1.72 (0.41) a	0.80 (0.24)	7.78 (4.1)	0	0	0.04 (0.04)	0
25°C	0.12 (0.92) a	0.12 (0.07)	0	0	0	0	0
All		0.12 (0.07)	4.37 (0.82)	0.05 (0.04)	0.08 (0.04)	0.01 (0.01)	67

* Means within a column followed by the same lowercase letter are not significantly different ($\alpha = 0.05$; Tukey's honestly significant difference test).
** Percentage of survival from stationary crawler to adult stage.

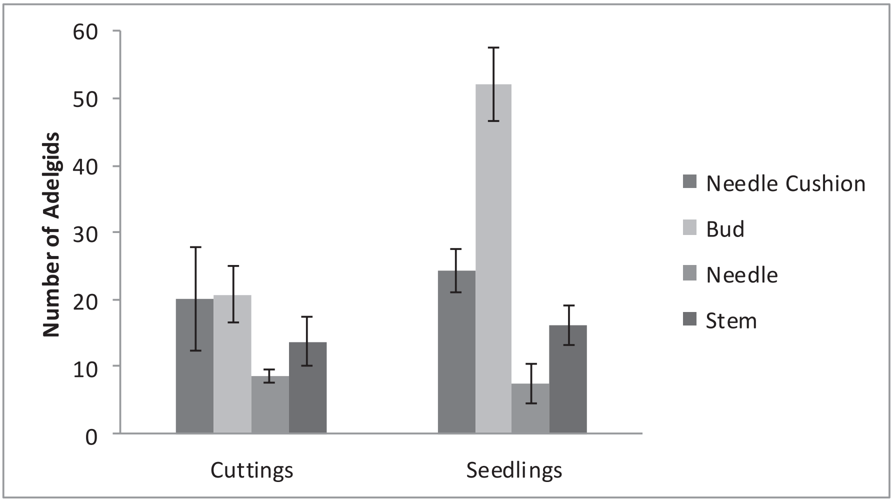

Fig. 1. Number of adelgids of all life stages inserting into Fraser fir across position.

Discs with medium texture had about twice the amount of inserted crawlers compared to discs with rough texture (Table 5), and all *A. piceae* that inserted into the bark developed into second instars. Texture clearly plays a role in *A. piceae* preference. A smooth or flat surface as well as too much roughness was unfavorable, whereas a medium texture with flat areas separating crevices and

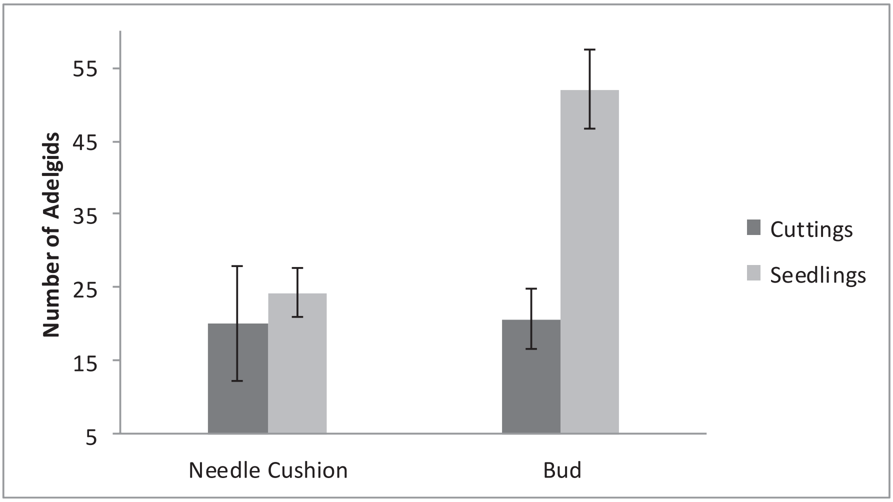

Fig. 2. Number of adelgids that reached full development on Fraser fir across position.

Table 3. Mean (±SE) number of all adelgid life stages per centimeter across all environments and sample type on 12 paired cuttings versus seedlings of Fraser fir.

Source	All Stages*
Environment	
17°C	14.55 (5.07) a
20°C	12.96 (5.07) a
25°C	0.27 (5.07) b
Plant sample type	
Cutting	3.02 (4.14)
Seedling	15.51 (4.14)

* Means within a column followed by the same lower case letter are not significantly different ($\alpha = 0.05$; Tukey's honestly significant difference test).

buds was most favorable. This is possibly because this surface type provides protection for *A. piceae* or provides better access to parenchyma cells. Recent studies have shown primary metabolites on the surface of the plant can be detected by insects after making contact with the plant (Lombarkia and Derridj 2008). Therefore, another possible explanation is a textured surface (crevices and buds) may provide a chemical cue for crawlers to feed and in the absence of texture the crawlers will leave in search of another food source.

The lower edge and side of the vertical bark discs and in or around crevices were the most favorable insertion sites for *A. piceae*. This suggests *A. piceae* shows positive geotaxis preference. The discs were a quick way to determine environmental preference, but because of their small size, they are not realistic for mass rearing *A. piceae*. Less than 20% of crawlers inserted in host material,

Table 4. Mean (±SE) number of adelgid life stages across all environments on Fraser fir logs (190.8 cm^2/log).

Environment	All Stages	Stationary Crawlers	2nd Instars	3rd Instars	Adults	Eggs
17°C	0.16*	0.07*	0	0	0.10*	0
20°C	0.13*	0	0	0	0.13*	0
25°C	0.07*	0	0	0	0.07*	0
Greenhouse	0.06 (0.04)	0	0.02 (0.02)	0	0.03 (0.2)	0
Insectary	0.27 (0.09)	0	0.05 (0.03)	0	0.21 (0.07)	0

* SE not presented; only one log was used in those treatments.

Table 5. Mean (±SE) number of settled adelgid crawlers and overall percentage of adelgids that inserted into Fraser fir bark discs in response to environment × orientation and bark texture.

Source	2^{nd} Instars	% 2^{nd} Instars
Environment × orientation		
17°C × horizontal	1.6 (0.45)	11.3
17°C × vertical	1.6 (0.36)	17.3
20°C × horizontal	1.4 (0.42)	14.1
20°C × vertical	0.8 (0.6)	3.4
Bark texture		
Rough	1.3 (0.14)	
Medium	2.3 (0.62)	
Smooth	0 (0)	

suggesting that rearing *A. piceae* will require a very large number for successful reproduction.

Due to the similarity of the setup, the three chambers can probably best be compared for appropriate temperature conditions for rearing *A. piceae*. The 20°C chamber appeared to be the optimal for development, whereas the 25°C chamber offered the least favorable environment. This could be due to several factors, including the combined temperature and RH in the 25°C chamber being too high for *A. piceae* and very low airflow in the chambers. The space was much more confined than in the insectary, and the low airflow could encourage more contaminants, both of which could lead to reduced success among rearing environments. The open space in the insectary allows for more airflow and more material to be tested and observed in one environment. This was also the only environment where *A. piceae* was able to develop into adults on every substrate.

The objective of this study was to determine the optimal environmental conditions for rearing *A. piceae* on natural host material. Future rearing of *A. piceae* in the laboratory should be on logs or seedlings in a large enough area to permit adequate airflow, set at 20°C and 75% RH, and infested material should be checked at closer intervals than was done in this study to confirm *A. piceae* development. Infested Fraser fir bolts continue to produce thousands of crawlers after being hung for a long period and will not need to be changed often.

Acknowledgments

The authors thank John Strider for help with travel to Avery Co. to collect samples, transport of samples to North Carolina State University campus in Raleigh, set up of the experiment, and suggestions for methods to keep the logs hydrated. We thank David Bednar for providing the seedlings for this experiment and assisting with setup and counting of seedlings.

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