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Hemlock Woolly Adelgid

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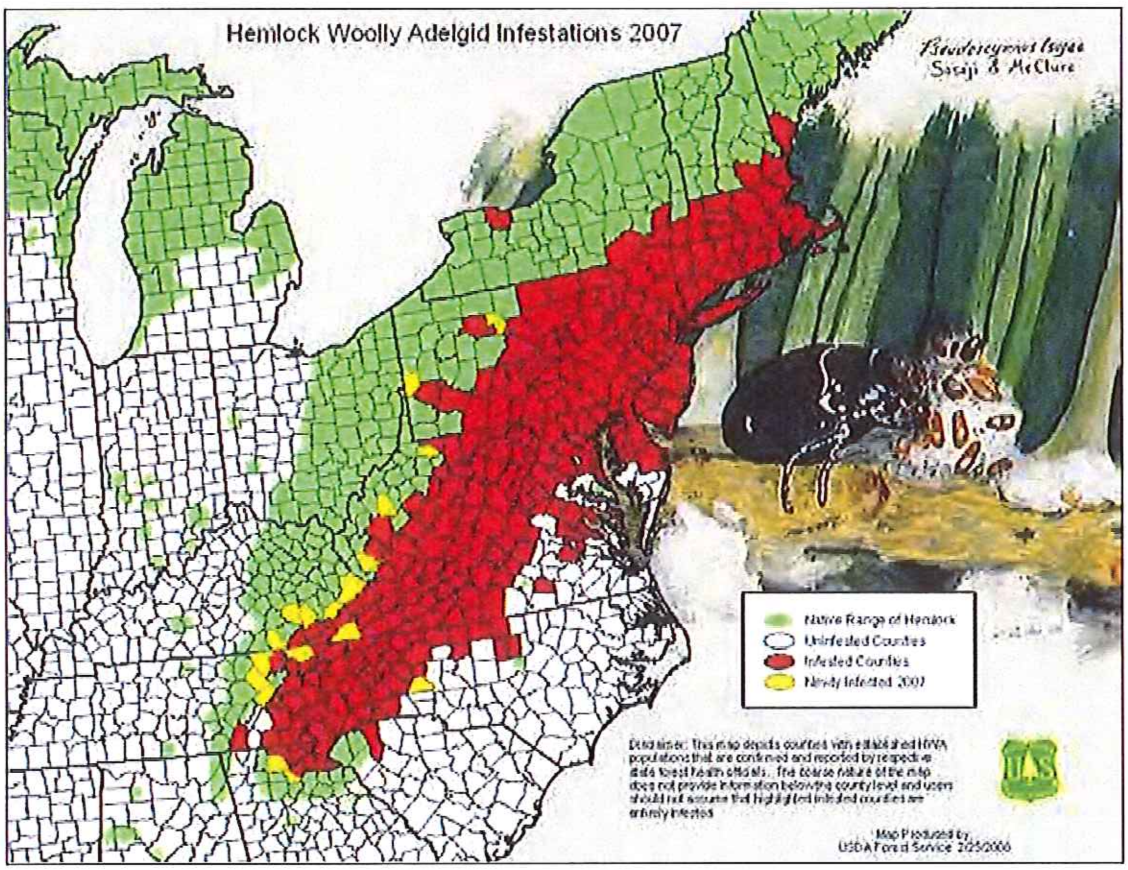

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Diet Development for Hemlock Woolly Adelgids and Their Predators

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ABSTRACT

We report here on progress in developing artificial diet-based rearing systems for hemlock woolly adelgids and one of their key predators, *Sasajiscymnus tsugae*. This report is divided into two parts: 1) summary of efforts to develop an artificial diet and diet-based rearing system for hemlock woolly adelgid (HWA), *Adelges tsugae* (Homoptera: Adelgidae), and 2) summary of efforts to develop a diet for adults and larvae of *S. tsugae* (Coleoptera: Coccinellidae). Efforts to develop a successful diet for HWA are complicated by complex and poorly understood feeding mechanisms and feeding targets. We offer evidence here for HWA feeding on particulate foods that are inherently more nutrient-dense than simple liquid solutions. Diet development for *S. tsugae* is somewhat complicated by the specific nature of adaptation to completing its life cycle on HWA. However, despite these complexities, we have succeeded in developing a diet that supports robust feeding by adults and larvae and which sustains high adult survival for over two months, allowing them to oviposit viable eggs with high levels of hatch (greater than 90%).

KEYWORDS

hemlock woolly adelgid, *Sasajiscymnus tsugae*, artificial diet, hemlock, biological control

HWA DIETS

Our earlier efforts to provide diets for HWA resulted in difficulties in eliciting robust feeding. We realized that much of the failure resulted from a lack of understanding of the fundamentals of feeding by these complex insects. Therefore, our recent research has been focused on developing a clear understanding of HWA feeding.

The first objective was to establish whether HWA uses particulate or liquid foods. We began our research on diet development under the assumption that HWA ingests liquids from parenchyma tissues of hemlock. However, failure to sustain HWA individuals on several liquid diets with complete nutrient profiles suggested that these insects may ingest foods that are not liquids. Observations of these facts confirmed our suspicions: 1) HWA and their close relatives balsam woolly adelgids (BWA) do not produce a copious excretory fluid as do aphids, whiteflies, leafhoppers, and other Homoptera that produce honeydew or a similar clear fluid that exceeds the insects biomass on a daily basis; 2) HWA guts (Figure 1) are not suited to concentration of dilute foods such as xylem sap, phloem sap, or other "plant juices"; and 3) extensive investigation of stylet placement in plant tissues by Young et al. (1995) and Cohen et al. (unpublished data) has led to unclear conclusions about the nature of feeding. Young et al. (1995) found that HWA stylets frequently terminated in parenchyma storage cells in xylem rays of hemlock needles (Figure 2). These authors contrasted HWA feeding with that of other adelgids and aphids that utilize as nutrients solutes in phloem. However, it is not clear from the study by Young et al. (1995) exactly which materials from xylem ray-parenchyma storage cells are ingested by HWA. We feel that, in light of our observations stated as 1) and 2) above, it is unlikely that a simple solution of parenchyma cell sap would be adequately concentrated to meet the growth needs of HWA. Unlike aphids (which feed generally on phloem sap) and leafhoppers (which feed generally on xylem fluid), HWA does not have the anatomical complexity of gut structures to accommodate the filtration and concentration processes evident in the two former groups of insects. Furthermore, the lack of copious excretory liquid suggests that HWA ingests a fairly, if not highly, concentrated plant material.

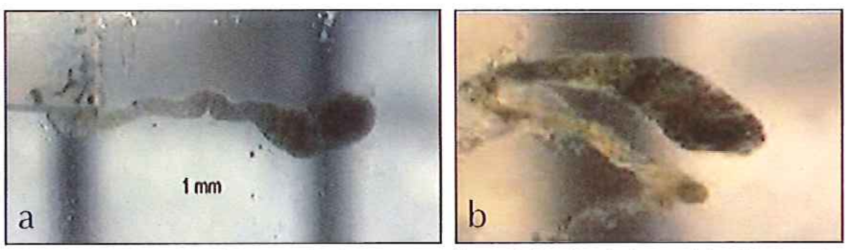

Figure 1. Guts from adelgids: a) from a balsam woolly adelgid adult and b) from a hemlock woolly adelgid adult. Note the simple structure of these guts—characteristic of insects that feed on concentrated nutrients rather than dilute nutrients such as plant saps.

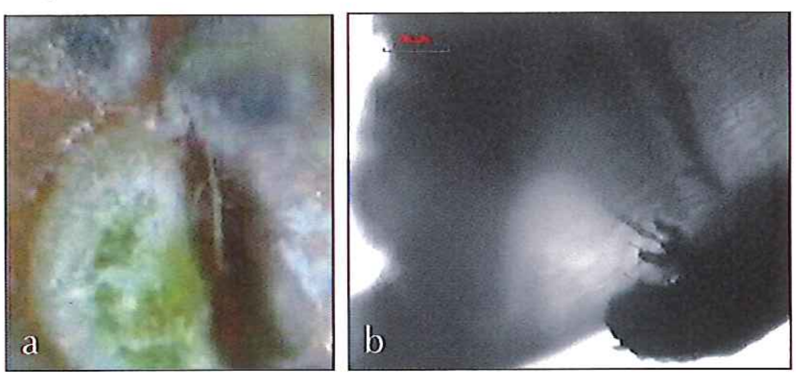

Figure 2. a) Adelgid stylet sheath in gap between hemlock needle and stem. This sheath (the T-shaped structure near the middle of the photograph) was started from the upper part of this image and moving toward the bottom. It can be seen to branch to the left and right. The green color of the needle is evident to the left of the branching stylet. b) Adelgid feeding on hemlock, forming a stylet sheath that is inserted into the needle base.

Thus far, we have had the best success in our diet trials when we provided HWA crawlers with a gelled diet composed of particulate materials such as Spirulina, *Arthrospira platensis,* and *Arthrospira maxima* (Phylum: Cyanobacteria). The Spirulina-based diets contain about 1-2% sucrose, 1-2% Gelcarin 812, 0.5% Vanderzant vitamins, 1-2% yeast extract, 0.1% Wesson salts, 0.2% sodium propionate, and 0.2% potassium sorbate. We have been testing these diets under the assumption that, for diets to be successful, they must meet the following four criteria (described in Cohen 2004): 1) palatability, 2) nutritional value, 3) bioavailability, and 4) stability. Simply put, the diet must elicit robust feeding and contain all growth and development requirements of the target species. The diet components must be absorbed by the insect's digestive system in appropriate amounts, and the diet must not spoil due to microbial growth, spontaneous oxidation, or enzymatic degradation.

SASAJISCYMNUS TSUGAE DIETS

Several introduced predator species from Japan, China and the Pacific Northwest are currently being reared for deployment in the HWA-infested states as part of the national biological control program supported by the USDA Forest Service. The mass rearing of *Sasajiscymnus tsugae* and other imported predators are completely dependent on extensive collections of healthy, heavily infested hemlock foliage. The availability of infested foliage is seasonal and can be unpredictable in quality. This dependence on natural prey collections and the necessity for labor-intensive techniques has placed numerical limitations on the large-scale production of specialist predators for adelgid control. Development of a predator artificial diet or dietary supplement for insectaries would greatly improve biological control efforts.

After testing numerous formulations (60 diets), a diet based on chicken eggs has shown excellent nutritive potential, and this, combined with a diet presentation system composed of uninfested hemlock foliage, has elicited good feeding response by adults and enabled survival of adults on diet alone for 8-10 weeks in 2006 while retaining reproductive capability. Research in 2006 has focused on new techniques for the production of a diet gel texture acceptable to *S. tsugae* that reduces congealment on mouthparts and allows the insect to traverse easily over the diet surface to feed. New cold-gelling techniques with sodium alginate and various calcium treatments were tested. The gelling of the base diet when dipped in a calcium solution forms a thin gel membrane around the liquid diet core, and beetles appeared to accept this formulation and were able to feed on it. This method of preparation has a preparatory advantage over the previously tested hot gel method using Gelcarin 812 as it does not require a heat source.

In 2006, *S. tsugae* readily fed on calcium-treated cold-gelled egg diet presented without hemlock foliage in Petri-dishes, but problems of desiccation resulted in diet globules drying up overnight. In early 2007, *S. tsugae* adult response to the egg-based artificial diet with calcium treatments on uninfested hemlock foliage was surprisingly much reduced. This was eventually found to be due to the inferior quality of the more recent freeze-dried formulations, which resulted in uneven dispersion of the diet particles in the hydrocolloid matrix. Equipment malfunction of the freeze drier resulted in an inferior base diet, which proved less attractive to *S. tsugae*. This behavioral observation also attested to the very specialized feeding preferences of this predator and the challenges of consistent diet formulation. However, cold-gelling techniques with sodium alginate appeared to provide an adequate protective skin around the diet that could be contacted without adhesion and penetrated by both larval and adult mouthparts.

In 2007, superior diets were produced for testing with *S. tsugae* adults. Extended and improved behavioral assays involved monitoring the adult feeding response and other behaviors in large 14 cm Petri-dish arenas (n = 5-10 adults/arena), which allowed for more natural, uncrowded behavior on 10-12cm hemlock tips. Adults are able to fly off the hemlock tips and move around the arena freely to exhibit real choice behavior. Ambient temperatures were also maintained between 23-25°C as adult *S. tsugae* show more activity and feeding behavior at these temperatures. Results showed that a non-gelled formulation of egg diet and honey preferentially attracted prolonged adult feeding when presented together with adelgid first instars just breaking dormancy and developing second instar nymphs (Figure 3).

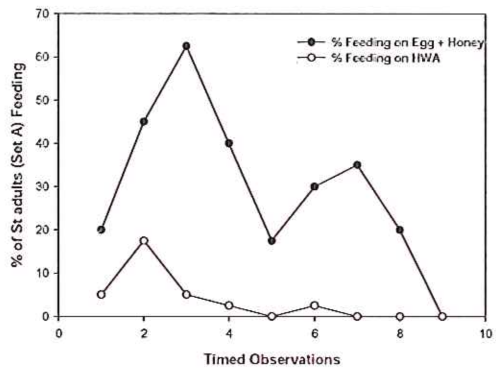

Figure 3. Preferential feeding by adult *S. tsugae* on egg diet and honey, given a choice of diet and HWA first and second instar nymphs.

Adults also were attracted over time (2 hours) to feed on dry egg diet powder mixed with honey and presented on filter paper in the absence of hemlock (Figure 4). This method was then tested as a supplement for maintaining long-term survival of adults on infested hemlock in holding cages kept at 14°C.

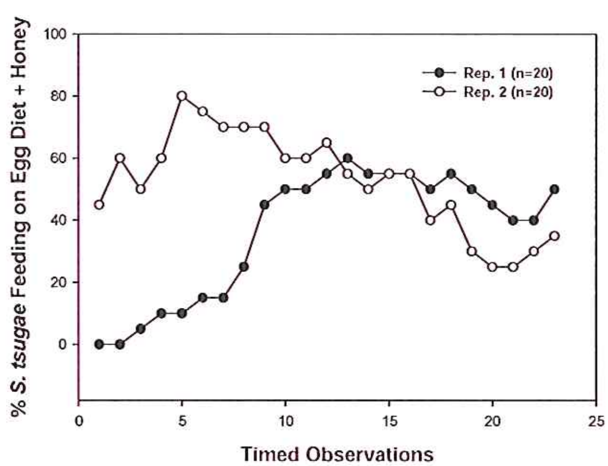

Figure 4. Mature adult *S. tsugae* response to egg diet and honey presented on filter paper.

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Results showed that, at 14°C, survival of mature adults in holding cages (n = 35-50/cage) with egg and honey supplement was superior to that in holding cages which had a supplement of Bug Pro© Gardens Alive beneficial insect supplement and honey (Figure 5). Survival on the latter supplement was unpredictable, while survival in cages with egg and honey supplement was consistently high even when holding hemlock material was desiccated and highly deteriorated with no live adelgids available for feeding. Mean percentage adult survival with egg and honey supplement was 85.1%, while mean percentage survival with Bug Pro© and honey supplement was 43.9%.

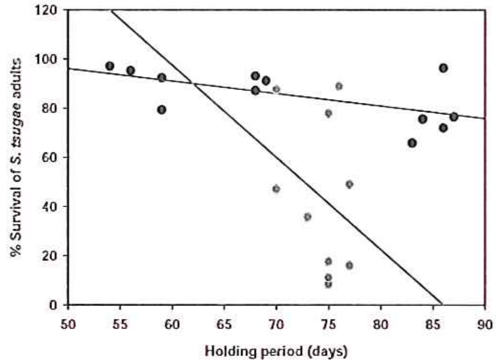

Figure 5. Comparative *S. tsugae* colony cage survival on adelgid-infested hemlock and supplements: 1) Cohen egg diet and honey (•) and 2) Bug Pro© Gardens Alive and honey (•).

Adults also significantly preferred gelled egg diet formulations over a pollen derivative diet in choice tests. Cold gelling of either diets produced an acceptable texture for both adult and larval feeding, but larval survival depended on having fresh moist diet available every two days as larvae are not attracted to dried diet and easily starve or desiccate to death. Mature larvae (third and forth instars) could survive for weeks with 50% survival at 17 days on diet alone but were unable to molt. This will be investigated further. Adults did not prefer gelled egg diets over second instar adelgid nymphs but fed equally on either HWA or diet by the second day. With larger HWA stages (third and fourth instars and adults), adults would start feeding preferentially on HWA, but by the third and fourth days, would switch to preferential feeding on the artificial diets—and on the egg diet in particular (Figure 6)—probably influenced by the depletion of adelgids with increased consumption over time. However, this behavior shows that *S. tsugae* will accept artificial diets for feeding when HWA is less available or of inferior quality.

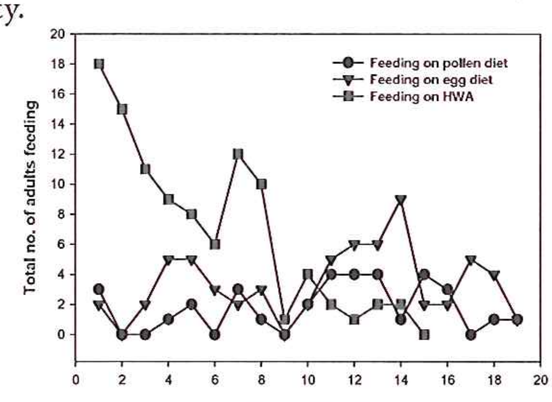

Figure 6. Change in adult *S. tsugae* feeding preferences with time when presented simultaneously with artificial diets and HWA.

Adults were then tested for their ability to survive on egg diet alone. Over 80% survival has been recorded at 8 weeks when adults have been maintained on gelled egg diet alone presented on uninfested hemlock, but frequent diet changes are necessary (every 2-3 days). Oviposition was also elicited when no live adelgids were present, but current observations indicate that an adelgid stimulant component is necessary, and this is being further investigated. Eggs that were laid on diet twigs were viable, with over 90% hatch.

RHEOLOGICAL PROPERTIES OF DIETS

One of our most novel discoveries in this work and related research is the importance of texture as a suitability factor of the artificial diets for both HWA and their predators. We have used a combination of cold-gelling agents to give a firm texture to the outside of the *S. tsugae* diet and gelling agents (or hydrocolloids) to give suitable viscoelastic properties to the diets. Besides viscoelasticity (G' and G" in Figure 7), there are several other features of texture that are proving to be important to the palatability of diets by insects, including *S. tsugae*. Figure 7 shows measurements made with rheological equipment testing four insect diets. Properties such as gumminess, hardness, fracture force, chewiness, and adhesiveness all seem to impact the "decision" of beetles to feed on or reject a given diet. We are currently trying to understand the range of values measured objectively with rheological techniques to determine the complex of textural characteristics that result in a highly palatable diet versus a diet that the insects reject.

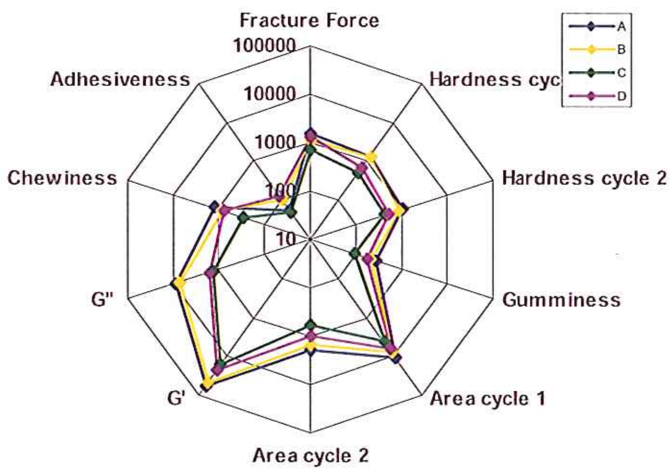

Figure 7. Diagram of rheological properties of four insect diets made with different gelling agents. The diagram illustrates the various texture qualities of diets, such as hardness, adhesiveness, chewiness, gumminess, and viscoelastic proportions (G' and G"). We have found these qualities to be greatly significant in determining the suitability of insect diets for rearing *S. tsugae*.

DISCUSSION

HWA

The exact nature of HWA feeding is not clear, but our work provides evidence that, like many Hemiptera, these insects may use extra-oral digestion where solid components of cells are pre-digested by the insects' salivary enzymes, then ingested a concentrated nutrient "broth." Our preliminary work with particulate diets based on algae and yeast provide some promise that particulates will be more useful than the strictly dissolved solute-based liquid diets that we have tested in the past. We have also made considerable progress in using flow-through systems to overcome the problem of diets becoming stale over the long feeding periods displayed by HWA. Our current efforts are intensely centered on more complete understanding of the nature of feeding by HWA and the exact feeding targets so that we can use these as physical and biochemical models for artificial diet.

Sasajiscymnus tsugae

We have succeeded in showing that *S. tsugae* adults will readily accept and feed on a gelled egg diet in the absence of HWA when the diet is presented on hemlock, with long-term survival on diet alone if diets are replaced regularly and frequently—every 2-3 days—to remove mold and maintain palatability and freshness. Shelf life of prepared diets is also very important as diets need to be formulated at a minimum of every 3 weeks and refrigerated. The texture provided by the gelling agents is very important as it allows adults and larvae to insert mouthparts to feed without interference and obstruction. Mature larvae will now readily feed for extended periods of time on fresh gelled diets. Premature desiccation of diets remains a challenge, especially for larvae, and will be the focus of future research.

ACKNOWLEDGMENTS

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