Chapter 14: Development of Artificial Diets for Predators of Hemlock Woolly Adelgids

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ABSTRACT

We report the results of the process that we undertook to develop a rearing system based on artificial diets or factitious prey for two species of specialist predators of hemlock woolly adelgid, Adelges tsugae (HWA). The predators in these studies were beetles (Coleoptera), *Sasajiscymnus tsugae* (Coccinellidae) and *Laricobius nigrinus* (Derodontidae). Besides testing more than 50 different artificial diets, we also attempted to use factitious prey, including insect eggs, insect larvae, and annelid worms. We also experimented with a variety of diet presentation systems that were designed to fulfill the feeding requirements of the two beetle species and to meet the needs to preserve the diets to prevent desiccation and deterioration. Although we had little success with most of the artificial diets and none of the factitious prey, we succeeded in developing several forms of a hen's egg-based diet and a diet presentation system that involved both gels made from alginate and slurry diets that were made from adhering liquid materials to a proprietary solid/capture medium. The most successful diets and diet-presentation systems allowed adults of both species of predators to stay alive and active for several months and to return to egg production after being returned to natural hosts (HWA) for a few days. Larvae fed readily on these chicken egg-based diets, but they failed to develop on any of the diets.

INTRODUCTION

Biological control of invasive adelgids remains one of the most promising means of control for these threatening pests. Predators have been the most emphasized biological control agents (Grenier et al 1994). Currently, rearing programs for two widely-used predators (*Sasajiscymnus tsugae* Sasaji and McClure, Coleoptera: Coccinellidae, and *Laricobius nigrinus* Fender, Coleoptera: Derodontidae) use hemlock woolly adelgids, *Adelges tsugae* Annand (Hemiptera: Adelgidae), collected from eastern hemlock, *Tsuga canadensis*. The collection of branches infested with adelgids is a costly process in terms of labor, travel, and destruction of large portions of the trees. Clearly, this method of rearing predators imposes severe limitations to the scale of production. A further complicating factor is that the complexity of the HWA life cycle (Figure 1) imposes further limitations on how many predatory beetles can be produced and even more constraints on the quality of the predators. Therefore, an artificial means of supplying high quality nutrition to HWA predators would be a tremendous advantage to HWA control programs.

When we specify "artificial nutrition", we imply either 1) insect prey that are not natural hosts to the predators, known also as factitious hosts, or 2) artificial diet that is composed partially or entirely of non-insect derived materials. Several species of predators (ladybeetles, pirate bugs and lacewings)

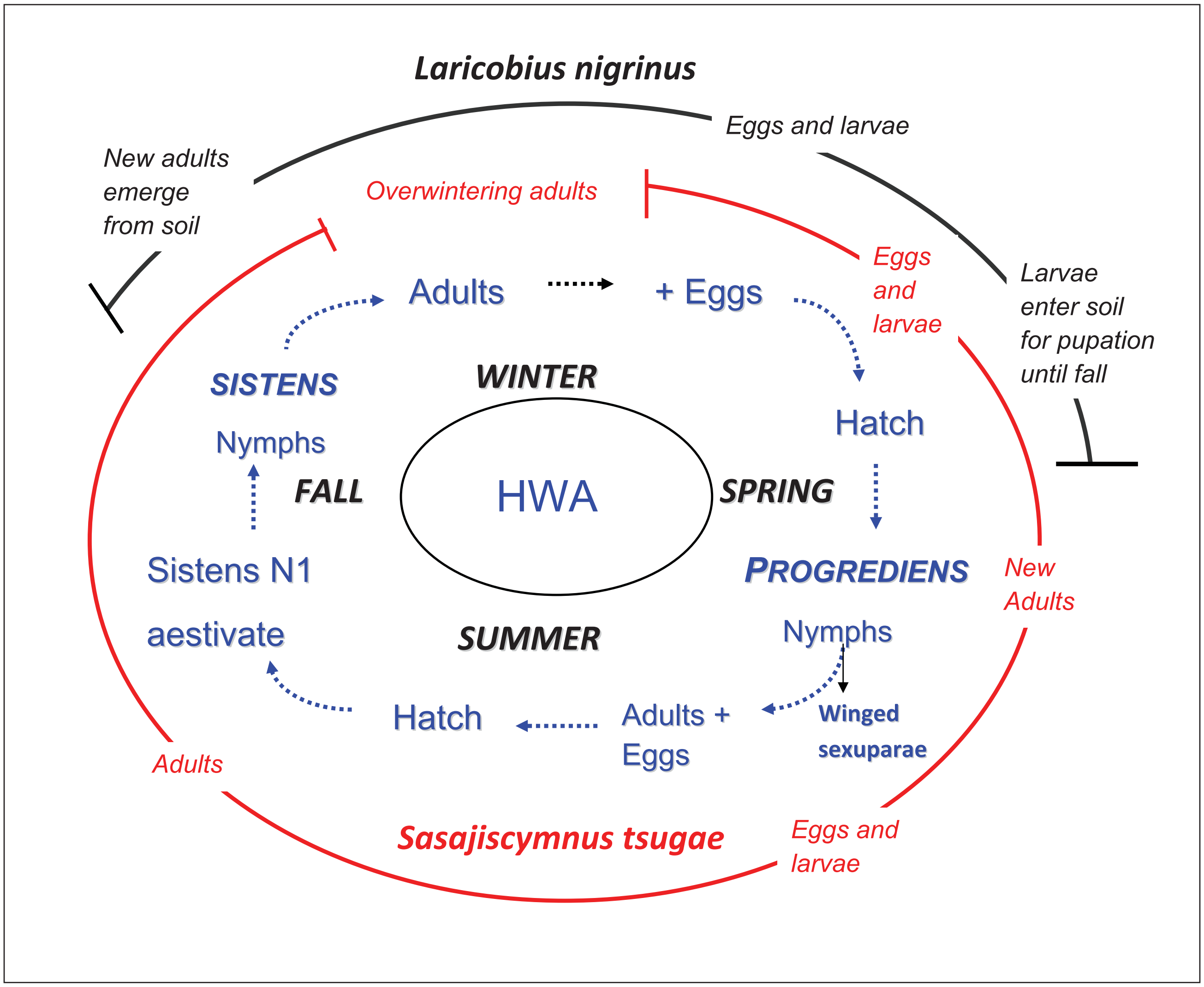

Figure 1. Life cycles of the hemlock woolly adelgid (------) and its introduced predators, *Sasajiscymnus tsugae* and *Laricobius nigrinus* in eastern North America. Solid lines indicate relative active periods of adult and larval predator feeding on the adelgid (*S. tsugae*; *L. nigrinus*); Cheah 2011.

and parasitoids (such as *Trichogramma*) have been reared successfully on factitious hosts such as the eggs of brine shrimp (Arijs and de Clercq 2001, Castane et al. 2006) and the eggs of various grain moths such as the Mediterranean grain moth *Ephestia kuehniella* (Pyralidae) (Bonte et al. 2010) and the Angoumois grain moth, *Sitotroga cerealella* (Gelechiidae) (Abdel-Salam et al. 2001). The advantages of using factitious hosts are that they are more conveniently available and generally less costly than natural hosts (Cohen 2003), but the disadvantages are that they are not always the most nutritious prey, and they are more expensive than artificial diets. Besides nutritional value and palatability, a further consideration in various foods used in mass-rearing is the packaging of predators' foods. The cuticle of natural and factitious hosts is a rather incredible packaging material, being made of chitin. While the cuticle is strong and water-proof, it can be as thin as 5-10 µM. This means that even predators with very small, short mouthparts can penetrate the cuticle and gain access to the foods. Furthermore, it is important to understand that many predators (including those in the current study) feed by extra-oral digestion where they inject digestive enzymes into the host to pre-digest it (Cohen 1990). The highly nutritious slurry that exists as a digestive product is then ingested, leaving behind the nearly intact cuticle empty of its previous contents. Also relevant to this discussion of packaging is the fact that the adelgids as prey are very small "packages" of nutrients (about 1-10 µg) which the specialist predators are fully adapted to feed on and thrive. Likewise, factitious prey, such as *Ephestia* eggs, are small (ca 20 µg), and they resist microbial contamination and chemical deterioration, in part by being so small and separate from one another, that contaminating micro-organisms do not spread through an egg mass. All this being said, it is clear that a mass rearing system for *S. tsugae* and *L. nigrinus* would be well-served if it could utilize factitious prey instead of natural hosts. In Figure 2 (a-d), *S. tsugae* are pictured feeding both on the natural prey (HWA) and artificial diet. In this case, the diet presentation is either in the form of natural eggs of HWA or the chicken egg diet offered as an alginate gel whose surface was made into a film by allowing the diet to interact with a calcium compound used as a cross-linking agent.

The description of natural and factitious prey leads to a comparison of the issues with artificial diets and the requirements of a diet presentation system for *S. tsugae* and *L. nigrinus*. As explained by Cohen 2003, for an artificial diet and diet-presentation system (called "diet system" for this chapter) to be considered completely successful it must:

1. Stimulate robust feeding
2. Support survival
3. Support growth and development
4. Support reproduction
5. Allow production of continuous generations indefinitely
6. Support high quality insects that are fully useful for their intended purpose (biological control, genetic pest management, food for other species, conservation, education, research, etc.)

Relatively few diet systems have been developed to meet all these specification, with a rough estimate of about 20 basic diets that have been shown to support about 300 species (Cohen 2003, Singh 1977). The accepted diets developed for *S. tsugae* and *L. nigrinus*, have supported survival in the absence of adelgids for several months (*S. tsugae*) and one month (*L. nigrinus*), with negligible mortality. Adults of *S. tsugae* fed exclusively on diet were able to commence normal reproduction on return to adelgid-infested foliage, although eggs were not laid while on diet only. Use of the most successful diets as supplements have improved laboratory survival of adult predators when quality and or quantity of adelgid-infested foliage was degraded or depleted.

Even fewer diets have been developed successfully for predaceous lady beetles (Kariluoto et al. 1976, Racioppi et al. 1981, Hodek 1996), the most notable success being that of Attalah and Newsom (1966), who reared eight successive generations of *Coleomegilla maculata* De Geer on a diet void of insect materials. Several authors reported using factitious diets such as formulations with powder honey bee brood (reported in Singh 1977).
The most successful factitious host diet for a coccinellid in our experience was the use of pink bollworm eggs to rear multiple generations of

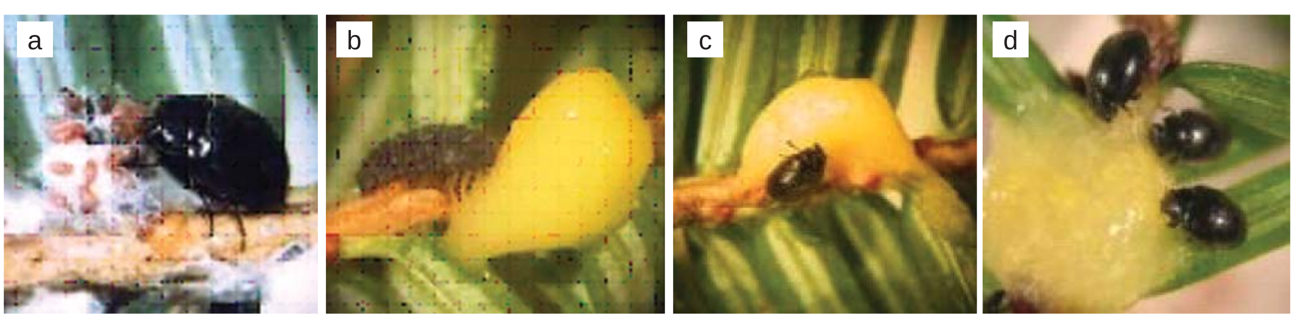

Figure 2. *Sasajiscymnus tsugae* (a) adult feeding on HWA egg sack, (b) larva feeding on artificial diet and (c & d) adult feeding on artificial diet (photos by C. Cheah).

Serangium parcesetosum in the USDA, APHIS Pink Bollworm Facility in Phoenix, AZ in the 1990s in a program developed by Dr. Robert T. Staten.

SPECIALISTS VS. GENERALISTS

In most programs and research efforts to control forest pests, specialist predators or parasitoids have been used in classical biological control systems (Pschorn-Walcher 1977). Because hemlock woolly adelgid is an exotic pest, only host specific natural enemies have been selected, such as the HWA specialists, *Laricobius* and *Sasajiscymnus*. In accord with the complex life cycle of HWA in the Northeast (Fig. 1), both species of predators have complex life cycles (Cheah and McClure 1998, 2000; Zilahi-Balogh et al. 2003), which track the prey's periods of dormancy, or reductions or surges in nutritional availability (Fig. 1). This "tracking" and "meshing" of the predators' life cycle with that of the prey is an indication of the degree of specialization of the predator. A further indication of the high degree of specialization is the fact that *L. nigrinus* has been shown to be restricted either nutritionally or in terms of feeding stimulation to require HWA to complete its life cycle. This fastidious feeding response, which excludes acceptance of substitute (factitious) prey, raises the question about whether rearing *L. nigrinus* can be possible on foods other than HWA.

The life cycle of the hemlock woolly adelgid is complex, with progrediens and sistens generations, along with phases where the insects actively feed, develop, and reproduce, and other periods when it is inactive, e.g., during summer aestivation. Along with the morphological and behavioral differences in HWA during these different phases, there are also biochemical differences. Our preliminary work on lipid, carbohydrate, and protein content indicates that during certain periods, HWA nutritional value greatly drops, especially during the late phases of their torpor and early stages of feeding activity onset. We have found the nutritional composition of HWA in their inactive phases drops to less than half of what is present in actively feeding individuals. This helps explain the periods of dormancy in predators that are specially adapted to feed on HWA, where their nutritional needs must mesh with their hosts. This concept of predator/prey ecological meshing is depicted in Figure 1.

We have divided our experiments and observations into three major categories to cover all aspects of our research on development of artificial diets and rearing systems for predators of HWA:

I. Feeding on Natural Hosts (Prey)
II. Feeding on Factitious Hosts
III. Feeding on Artificial Diet
   a. Various artificial diets
   b. Diet presentation techniques
   c. Factors in diet stability

Natural Hosts

The different life stages of HWA offer different nutrient rewards to predators, especially with respect to 1) overall biomass, 2) protein content, 3) lipid content, 4) carbohydrate content, and 5) vitamins and minerals. We have begun analysis of these factors in several of the life-stages, but because these studies are preliminary, we can provide only partial results. Using the analytical techniques outlined in the chapter (Chapter 13 in this volume), we have found that eggs that have been oviposited, as well as eggs that are inside females, have a high lipid content, with approximately 50% of the dry weight of an egg being lipid. The protein content ranges from about 30-40% of dry weight and the carbohydrate content is less than 10%, leaving about 4-5% ash (minerals) and a small biomass (less than 3%) composed of other components such as nucleic acids and components derived from host plants. These findings are in accord with Cohen and Patana (1985) regarding the nutritional composition of eggs. However, adelgid eggs and neonate larvae have a higher lipid content than comparable lepidopteran eggs and neonates.

It is evident from Figure 3 that a great deal of lipid material, especially oil, is stored in the eggs and remains present in the neonate crawlers. The oil is present as a storage material providing

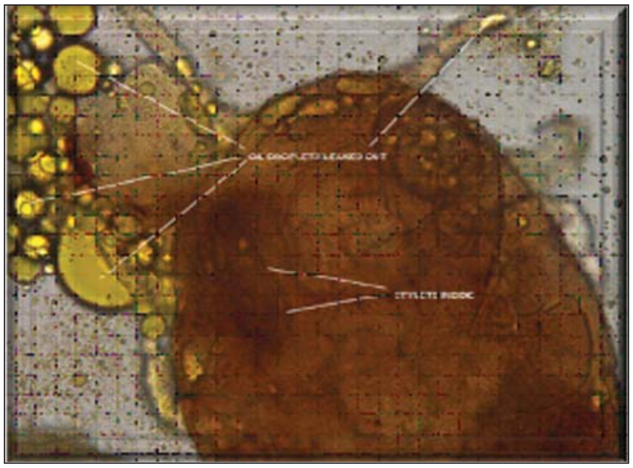

Figure 3. A pre-hatched 1st instar adelgid nymph that had a gentle pressure applied to express the droplets of oil that are present inside the insect (photo by A. Cohen).

energy and bio-materials for crawlers as they settle on host plants and begin their feeding forays. The lesson that we learn from analysis of natural prey is that HWA predators, especially those that feed on adelgid adults with eggs, derive a large lipid component from their diet.

Factitious Prey

The use of factitious prey or hosts has a long history in rearing entomophagous insects. For example, the Mediterranean grain moth *Ephestia kuehniella* (Pyralidae) and the Angoumois grain moth *Sitotroga cerealella* (Gelechiidae) have been used extensively in commercial predator and parasitoid production of green lacewings and egg-parasites such as *Trichogramma* spp. (Cohen and Debolt 1983; Cohen and Smith 1998).

In Figure 4a, an egg that had been fed on by *S. tsugae* is seen with its characteristic depletion of materials removed by the predator. The egg is also dark as a result of the polyphenol oxidase action that takes place during the extra-oral digestion process (Cohen 1995). Because *S. tsugae* fed minimally on *Ephestia* eggs, we also tried to “disguise” the *Ephestia* eggs by placing fresh adelgid wool and exudates around the eggs, to determine whether or not the HWA materials would enhance predation. All uses of factitious prey met with little success and a very limited amount of feeding on such prey.

Feeding on Artificial Diet:

a. Various artificial diets

Starting with the Cohen and Smith 1998 diet, we tested more than 100 different diets. The formulations that we have deemed most successful are proprietary combinations of cooked chicken egg mixture with functional diet components that are suspended in a freeze-dried (proprietary) carrier material. The formulation is again freeze-dried and stored until used with appropriate re-hydrating agents, which are discussed below (under “diet presentation techniques” and “diet preservation or stability techniques”).

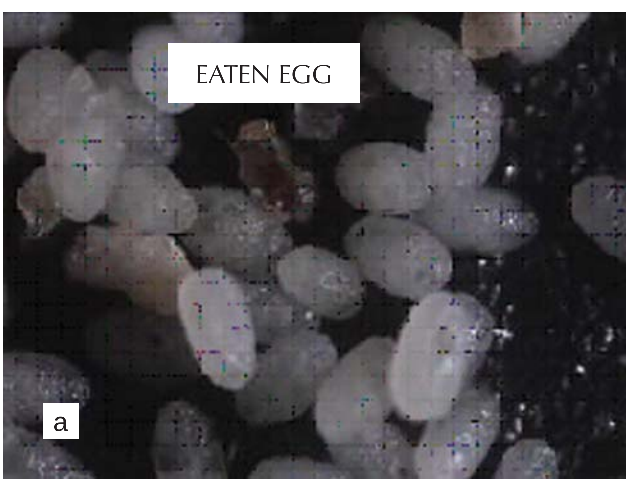

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Figure 4. Eggs of Ephestia kuehniella that were exposed to Sasajiscymnus (a) and Laricobius (b).

b. Diet presentation techniques

It has long been realized that diet texture and presentation techniques can be as equally important as the nutritional composition of the diet itself (Singh 1977, Cohen 2003). Diet researchers have often resorted to the "missing nutrient hypothesis" (Cohen 2003) to explain the reasons for a diet's failure. This hypothesis was advanced in early studies of insect nutrition by such eminent researchers as G. Fraenkel and S. Beck, who had discovered cryptic nutrients that were essential factors for insects as a whole or target species. For example, the discovery that sterols were essential to insects went far to explain why earlier formulations that lacked sterols failed to support insect growth. Similarly, the discoveries of carnitine and choline as essential to some insect species further supported the "missing nutrient hypothesis". However, extensive analysis and empirical trials during many diet-development studies did not reveal hidden nutrients that could result in successful artificial diets for a large number of insect species. Such failures may very well be explained by alternative hypotheses such as texture failure or packaging (or presentation) failure. For example, if an insect uses extra-oral digestion, presentation of a complete diet that is in liquid form can be unsuitable (Cohen 1985, Cohen 1995, Cohen and Smith 1998).

Other issues in diet presentation include whether 1) the diet is covered with a material that can be penetrated by the target insect's mouthparts, 2) the diet covering is capable of preventing excessive water loss or other degradation factors such as contamination with microbes. In response, diet researchers have used films such as Parafilm™ or Whatman Laboratory Film, the dipping of diet in molten wax, or encapsulation techniques such as those illustrated in Figure 5.

Some feeding responses of *S. tsugae* are shown in Figures 6 through 8. *Laricobius nigrinus* adults also appear to accept similar diet formulations originally developed for *S. tsugae* (Fig. 9).

c. Factors in diet stability

Once a palatable and nutritious diet has been developed and the presentation system (encapsulation, film, uncovered diet, etc.) has been established, the next stage concerns the preservation or stability of the diet. In addition to using coverings that protect the diet from contamination, techniques that employ heat, water activity, extremely low or high pH, and/or chemical prophylaxis are conventionally employed.

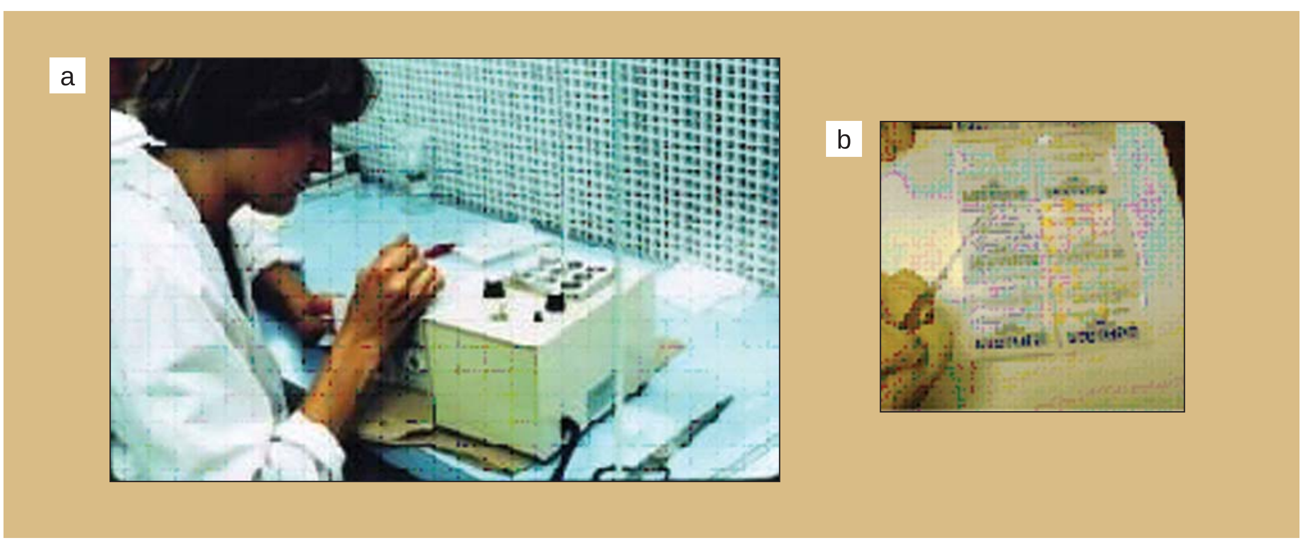

Figure 5. A technician (a) making wax-coated capsules (b) with liquid/slurry centers (according to the method of Cohen 1983; photos by A. Cohen).

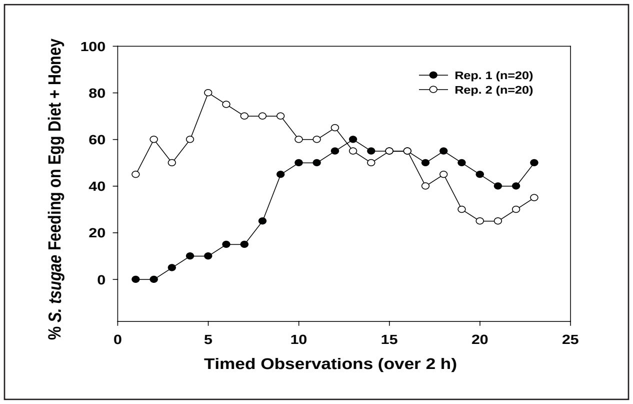

Figure 6. Mature adult *S. tsugae* feeding response to egg diet and honey presented on filter paper.

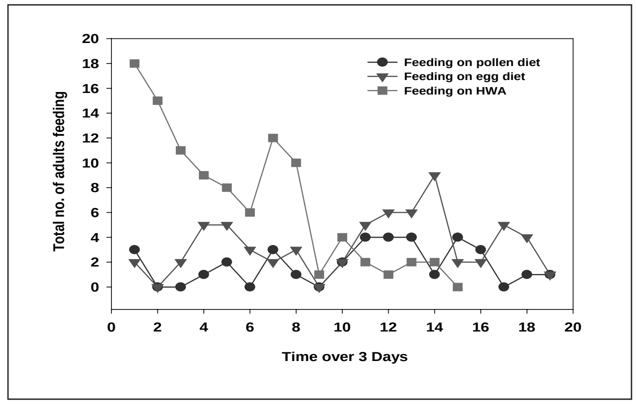

Figure 7. The changes in adult *S. tsugae* feeding preferences over time when presented simultaneously with artificial diets and HWA.

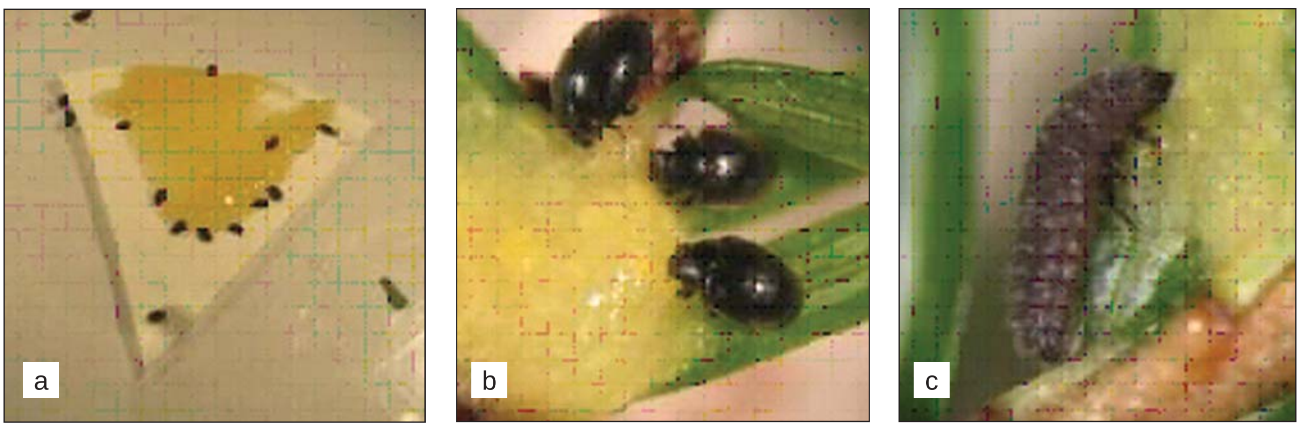

Figure 8. *Sasajiscymnus tsugae* adult and larval feeding on an egg-based diet which has been mixed with honey or an alginate/calcium base. The diet was presented on (a) filter paper with honey or (b) as an alginate formulation on hemlock twigs; (c) adults and late instar larva (photos by C. Cheah).

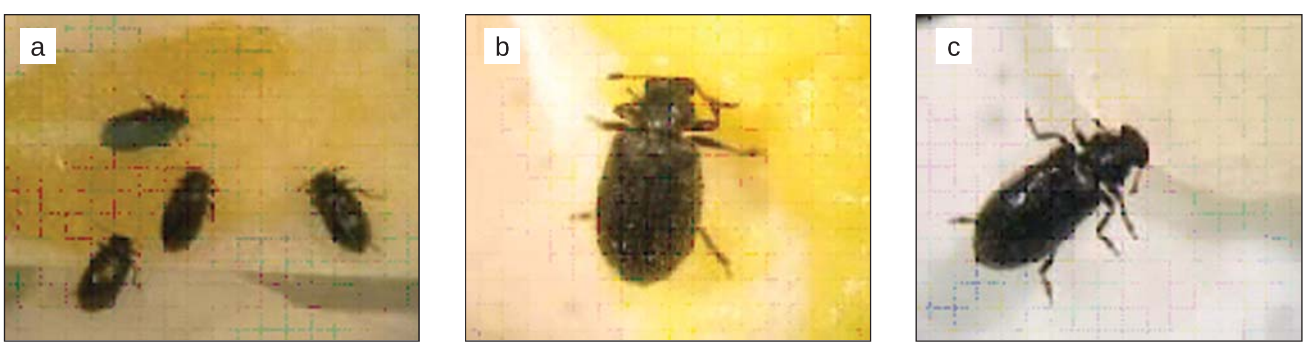

Figure 9. *Laricobius nigrinus* adults feeding on (a) egg diet mixed with honey, (b & c) egg diet-alginate formulations (photos by C. Cheah).

The use of heat is nearly universal in diet synthesis where diets are heated to the point where all microbes are killed (by autoclaving or other temperature/pressure treatments) or where vegetative stages of microbial contaminants have been killed (Pasteurization). We have found that either method produces diets that can be made acceptable to the predators in terms of maintaining palatability. We have demonstrated this fact with the egg-based formulation that we call FDFE3 diet. However, because the predators can inoculate the diet with microbes while they are feeding, heat treatment alone cannot continuously protect the diet from microbial contamination. Therefore, we resorted to lowered water activity and chemical preservatives.

The concept of water activity (a_w) is a useful way of expressing the tendency of a diet to suffer microbial deterioration and other degradation processes such as oxidation. Water activity is expressed as a unitless ratio of the actual vapor pressure divided by the potential vapor pressure at a given temperature. This can be conveniently thought of as relative humidity divided by 100. Common examples of foods with naturally low water activity are honey and molasses. Although both of these are liquids at room temperature, and although both contain ample nutrients, neither supports microbial growth. Water activity below 0.60 does not support microbial growth of any of the common environmental microbes that habitually contaminate our diets. The range of water activity is between 0 and 1.0, with the lower value being associated with concentrated sulfuric acid and the higher number being associated with distilled water. Most common diets for insects, including most of those that we have tested, have a water activity of 0.98-0.99 (Cohen 2003). This means that the diets are well above the minimal threshold for support of microbial activity, and unless we used special measures to lower water activity, we must expect the diets to be subject to unimpeded microbial growth. In contrast with common insect diets, honey has a water activity of about 0.50-0.55; therefore, it is very uncommon for microbial growth to take place in honey.

Therefore, we have incorporated several measures to lower the water activity of our diets, including the use of freeze drying diets for preservation, then hydrating the diets with honey or similarly low water activity hydrating sources (such as glycerol or sorbitol solutions). We have found honey to be an especially suitable medium for presentation of diets to adult *S. tsugae*. In keeping with our efforts to lower water activity of test diets, we have used freeze drying (lyophilization or cryodesiccation) to prepare diets for storage, transport, then re-hydration with appropriate mixtures to help retain low water activity. We have adopted freeze drying extensively because it not only preserves diet components that are highly perishable (such as eggs), but it also lends itself to diet presentation techniques that maintain low water activity. To clarify this concept, we offer this example. If we lower the water activity of our egg diet from 0.98 to 0.10 by freeze drying, then if we re-hydrate the diet with honey (water activity of 0.50), the overall diet/honey mixture has a water activity of 0.50 or less. This mixture will not support microbial growth, and it has an extremely long shelf life (well over 6 months without refrigeration). We must add that the concept of lowering water activity with materials that have high concentrations of dissolved small molecules (such as sugars, sugar alcohols, or salts) is known as the "***humectant***" strategy.

However, the two drawbacks of the use of humectants is that 1) the material making up the humectant/water mixture may be unpalatable to the target insects (for example, glycerol is not palatable to the predators thus far tested), 2) the mixture can be very sticky and cause insects to become trapped (we have found this with *S. tsugae* larvae), and 3) the humectant/diet mixture can become hydrated (for example, by watering the cage contents to provide free water to the predators or to raise the humidity), and this hydration can raise water activity to above the minimal threshold to prevent microbial growth.

This leads to the 3rd strategy: the use of low pH. We have used various pH lowering agents such as acetic acid, propionic acid, phosphoric acid, and citric acid (all fairly well-established in insect diets) to lower the diets' pH below 5.0. We have found that acetic acid and citric acid are fairly well-tolerated by the predators, and they are fairly, but not totally, efficient at lowering microbial growth. For example, we have found little problem with bacterial contamination and deterioration of our diets, but fungal contamination remains a problem in our formulations.

Therefore, we have had to resort to the 4th strategy of using chemical agents to prevent fungal growth. We have experimented with several anti-fungal chemicals (benzoic acid, nystatin, methyl paraben, sodium propionate, and potassium sorbate, to mention a few), and we have discovered that the most well-tolerated agents are propionate and sorbate in their salt forms, which makes them soluble in our diets. We also have found that the combination of the acids and the antimicrobial chemicals have added to the efficiency of mold-prevention and the duration of the time frame that diets can be kept in cages. However, we must add that because of the highly nutritious character of the diets, they still attract mold growth after several days or several weeks' exposure to the predators. Therefore, mold prevention remains one of the barriers to completely successful diets for HWA predators.

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