

## **Rearing *Hylobius transversovittatus* and *Cyphocleonus achetes* Larvae on Artificial Diets (Coleoptera: Curculionidae)**

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REARING *HYLOBIUS TRANSVERSOVITTATUS* AND *CYPHOCLEONUS*  
*ACHETES* LARVAE ON ARTIFICIAL DIETS  
(COLEOPTERA: CURCULIONIDAE)

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*Hylobius transversovittatus* Goeze and *Cyphocleonus achetes* (Fahraeus) (Coleoptera: Curculionidae) are large weevils that share many life history traits. Adults feed above ground on the leaves and shoots of their larval host plants. Females oviposit in stems or roots, and larvae burrow into the roots where they feed, complete larval development, and eventually pupate. *Luthrum salicaria* L. and *L. alatum* Pursh (Lythraceae) are host plants for *H. transversovittatus* (Blossey 1994), and *Centaurea maculosa* L., and *C. duffusa* L. (Centauraceae) are the primary hosts of *C. achetes* (Stinson et al. 1994). These weevils are known to have a narrow host range and are agents for biological control of weeds.

Our initial studies on the development of artificial diets for root-feeding biological control agents began with adaptations of the Gast diet (Robertson & Wright 1984). We formulated a fully functional diet for *H. transversovittatus* that yielded adults with a 35% survival rate (Tomic-Carruthers 2007). Here we present an improved diet and handling system that increased *H. transversovittatus* survival, and extended use of the diet for other insects.

Most of the ingredients in our diet were industrial grade products obtained from Bio-Serv or directly from the producers. Pharmamedia® (Traders Protein, Memphis, TN) and common chemical ingredients were ordered from the Sigma-Aldrich Company. The diet was prepared as previously described (Tomic-Carruthers 2007) except for several modifications to facilitate and simplify the process. The improved diet was mixed in 1-L batches with a Cusinart Pro Classic household food processor. Dry ingredients (Table 1) were mixed thoroughly in the food processor with the exception of the vitamins, antibiotic, and agar. Half of the required volume of tap water (65°C) was added to the mixture and blended for 3 min. The remaining water was combined with agar and heated to boiling in a microwave oven before it was added to the mixture in the food processor and blended for 30 s. The vitamins and Aureomycin (5.5% active) then were added to the mixture at 60°C, followed by blending for another 2 min. Finally, corn oil and HCl were added and blended for 30 s. The prepared diet was transferred to an automated biscuit maker (Atlas) and dispensed into rearing containers. Uncovered containers were left in a clean air bench for 3 h to cool before covering. Sealed cups were refrigerated until eggs or larvae were added.

TABLE 1. INGREDIENTS FOR THE ROOT WEEVIL DIETS.

Ingredients <sup>1</sup>	<i>Hylobius</i>	<i>Cyphocleonus</i>
Dry (g)		
Sucrose	15.9	32.0
Pharmamedia	63.5	32.0
Isolated soy protein	22.7	11.0
Wesson's salt	2.5	2.5
Cholesterol	0.7	0.7
Ascorbic acid	1.7	1.7
Potassium sorbate	1.1	1.2
Methyl paraben	1.6	1.6
Cellulose	150.0	150.0
Vitamin mix	2.5	1.5
Auromycin 5.5%	1.0	1.0
Agar	22.0	22.0
Liquid (mL)		
Water	860.0	900.0
Corn oil	1.9	1.8
HCl 1N	0.8	0.9

<sup>1</sup>(1 L Final volume).

Our original diet formulation for *H. transversovittatus* (Tomic-Carruthers 2007) was improved through a series of experiments on its nutrients and physical properties. Changes to protein and sterol concentrations did not increase yields; however, significant improvements in insect survival were achieved by augmenting cellulose and agar levels. This modification made the diet harder and dryer. We also changed the rearing containers to fit the biological requirements of the insects more closely. Originally, the diet was poured into rearing trays that contained 32 individual tubs (4 × 4 × 2.5 cm, l:w:h) (BIO-RT-32 from CD International, Bio-Serve). This provided an adequate amount of diet for each insect, but was problematic as early instars often burrowed to the bottom of the dish and died. To resolve this problem, 30-mL Cometware® (www.instaware.com) plastic portion cups (2.5 cm bottom, 4 cm top diameters, and 4.5 cm h) were tested. An experiment was performed in which 22 ± 3 mL of diet was dispensed into each of 30 individual cups versus 32 tubs (10 replicates). An individual egg was placed on the diet surface in each container and allowed to hatch and tunnel into the medium held at 25°C. After 130 d, each dish was examined to determine survival or instar at death. After eliminating con-

tainers with un-hatched eggs, the sample size was  $n = 550$  for pooled replicates (281 tubs and 269 cups). Adult yield was 63% in cups and 26% in tubs ( $P < 0.0005$ ; binomial one-tailed test, SPSS 14.0). This improved yield was a consequence of increased diet depth in cups versus tubs (4 cm vs. 2.5 cm). Deeper diet cups allowed neonate larvae to tunnel downward longer and establish in the diet before reaching the bottom of the container. Combining these physical improvements with changes in diet formulation (Table 1) eventually resulted in 85% survival of *H. transversovittatus* from neonate larvae to emerging adults.

Further modifications of the diet and rearing system facilitated the production of both *H. transversovittatus* and *C. achetes*. Although the *C. achetes* diet is still under development, 9 generations of this root-feeder have been successfully reared with slight variations. First instar mortality was initially a problem with this diet, as it was with *H. transversovittatus*, but depth of the diet was not the cause. The majority of dead neonate larvae were found on the diet surface or between the container wall and diet. Establishment and survival of *Cyphocleonus achetes* was improved by shredding solidified diet with a food processor before putting it into rearing containers. Shredding increased yields from 10-20% to approximately 40-50%, exceeding the survival of a previously developed diet based on wheat germ (Goodman et al. 2006). Presented diets differ significantly in the protein to sugar ratio (5.3:1 *Hylobius* vs 1.3:1 *Cyphocleonus* diet, respectively, Table 1). The requirement of *H. transversovittatus* for significantly higher protein levels in the larval diet is very likely associated with adult longevity. *Hylobius transversovittatus* adults can live several years (McAvoy & Kok 1999), while *C. achetes* lives only 1 season.

#### SUMMARY

A new meridic diet was developed to support the development of 2 beneficial root-feeding weevils important for biological control of invasive weeds. Although this diet is already in use in multiple insectaries for mass production of *H. transversovittatus*, it and the associated rearing system have not been published. In addition to the pri-

mary purpose for the diet, it has been used to rear the red palm weevil, *Rhynchophorus ferrugineus* Olive. Another potential use is for rearing of various root-feeding larvae of Coleoptera and Lepidoptera for biological control (M. Cristofaro, personal communication, E-mail: mcristofaro@casaccia.enea.it). The diet may support development of a number of other root and stem feeding insects.

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