An Artificial Diet for Cottonwood and Imported Willow Leaf Beetles (Coleoptera: Chrysomelidae) and Comparative Performance on Poplar Foliage[1, 2]

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ABSTRACT An artificial diet was developed for laboratory rearing of the cottonwood leaf beetle, *Chrysomela scripta* F., and the imported willow leaf beetle, *Plagiodera versicolora* (Laicharting). To reduce microbial contamination of the media, procedures were developed for separating egg masses and sterilizing egg surfaces. Cottonwood leaf beetle larvae reared from neonate to adult on this artificial diet had greater mortality, took longer to develop, and were smaller than larvae reared on fresh poplar foliage. Adult longevity was similar for both diet-and foliage-reared larvae. Survival and adult fresh weight of imported willow leaf beetle larvae reared on the artificial diet were similar to those of cohorts reared on fresh poplar foliage. However, individuals reared on artificial diet took longer to develop and produced shorter-lived adults than cohorts reared on foliage. Larvae of both species would not eat fresh foliage after being fed on the artificial diet. Adults of both species maintianed on the artificial diet laid few eggs but resumed normal oviposition when fed fresh foliage. This artificial diet proved useful for rearing larvae and maintaining adults during periods when fresh foliage was limited.

KEY WORDS *Chrysomela scripta, Plagiodera versicolora,* artificial diet, *Populus,* cottonwood leaf beetle, imported willow leaf beetle, Coleoptera, Chrysomelidae.

The cottonwood leaf beetle, *Chrysomela scripta* F., and the imported willow leaf beetle, *Plagiodera versicolora* (Laicharting), are multivoltine defoliators of *Populus* and *Salix* spp. (Baker 1972). Maintaining a laboratory colony of leaf beetles is labor intensive and requires large quantities of fresh foliage for food throughout the year. In addition, variability in leaf-to-leaf nutritional quality may introduce significant sources of experimental error in studies seeking to quantify the impact of specific variables on insect performance. Artificial diets greatly facilitate insect research by reducing costs and by minimizing variability in nutrition. This is particularly important for standardizing insect bioassays (Singh and Moore 1985).

The purposes of this study were (1) to formulate an artificial diet and develop laboratory rearing procedures for the cottonwood leaf beetle and the imported willow leaf beetle, and (2) to compare the responses of insects reared on the artificial diet to cohorts reared on poplar foliage.

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[2] Mention of a product name does not constitute an endorsement or a recommendation for its use by the USDA.

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Materials and Methods

Colony and Rearing Conditions. In the spring of 1988, cottonwood and imported willow leaf beetles (adults and eggs) were collected in Michigan from field-grown poplar and willow trees, respectively. These insects were used to establish foliage-fed laboratory colonies of both species. Insects were reared in plastic crisper boxes (20 × 10 × 13 cm and 20 × 8 × 9 cm) at 24 ± 1°C with a 16:8 (L:D) photoperiod. They were given fresh poplar foliage every 2-3 d. Egg masses were collected every 2-3 d by cutting a small area of leaf around each egg mass. Eggs were either used immediately or stored for later use in plastic crisper boxes with a moistened paper towel at 10 ± 2°C for up to 10 d.

Egg Sterilization. Beetle eggs were surface sterilized to avoid contamination and subsequent degradation of the artificial diet. Before sterilization, eggs were removed from the leaf surface using a paint brush dipped in 0.75% NaOH solution (Retnakaran and French 1971). Unlike willow leaf beetle eggs, which are laid individually, cottonwood leaf beetle eggs, are laid in tight aggregates that needed to be separated from each other prior to sterilization. Sodium hydroxide, at concentrations of 0.5, 0.55, 0.6, 0.75, 0.8, and 1.0%, was tested for separation efficiency of eggs within egg masses and impact on egg hatch. Sterile distilled water was used as a control. Separation was achieved by agitating solutions containing egg masses at 1,400 rpm on an orbital shaker for 5 min. Each NaOH concentration was replicated five times using 1-2 cottonwood leaf beetle egg masses ($\bar{X}$ = 40.4, SE ± 3.3 eggs per mass).

A method of surface sterilizing the eggs was determined by transferring the eggs with a Pasteur pipette in a sterile hood to various concentrations of disinfectants for different time intervals (Sikorowski and Goodwin 1985). The disinfectant treatments included 1-min exposures to bleach (5.25% sodium hypochlorite) at 5 concentrations (0.05, 0.3, 0.5, 1.0, 2.0%), and 4 concentrations of Zephiran Chloride™ (0.03, 0.05, 0.1, 0.2%). Treated eggs were counted and incubated on nutrient agar (2%) or filter paper in 100 × 15 mm petri dishes at 35°C for 7 d. Dishes were observed every 24 h for contamination and egg hatch.

Larval Rearings. Disinfected eggs were transferred again, with a minimum of water, to a 100 × 15-mm petri dish lined with sterile filter paper. The filter paper was allowed to dry until slightly damp before closing and sealing the petri dish with Parafilm™. Petri dishes containing eggs were placed in a plastic crisper with a moistened paper towel at 24 ± 1°C until eclosion.

Neonates were transferred to the artificial diet at a density of five larvae per cup using a small paint brush that had first been dipped in 0.2% Zephiran Chloride™ and then rinsed in sterile distilled water. Exuviae and dead larvae were removed with a sterile loop every 2-3 d. It was not necessary to transfer larvae to fresh cups of artificial diet during the larval period.

Artificial Diet. The artificial diet (Table 1) was formulated from the outcome of dietary trials using deletions and gradient concentrations of poplar foliage, oatmeal, wheat germ, casein, sucrose, or dextrose ranging from 0% to 30% (w/v) in 5% increments. In addition, recipes were modified with Sure II or Wesson's salt mixes, potassium hydroxide, and sodium ascorbate at concentrations ranging from 0% to 6% (w/v) in 1% increments.

Table 1. Composition of artificial diet for the cottonwood and imported willow leaf beetles.

Group	Component*	Amount
A.	Bacto-agar (Difco)	10.0 g
	Distilled water	500.0 ml
B.	Raw wheat germ (ground) (ICN)	10.0 g
	Rolled oats (Quaker Old Fashioned)	15.0 g
	Vitamin-free casein	20.0 g
	Lact-albumin hydrolysate (ICN)	6.8 g
	Dextrose	20.0 g
	Brewer's yeast (deactivated)	5.0 g
	Sure salt mix 2	3.0 g
	B-Cholesteryl chloride (Sigma)	0.4 g
	Cholesterol	1.0 g
	Choline chloride	0.7 g
C.	Vanderzant's vitamin mix	5.0 g
	Populus leaf powder†	15.0 g
	Raw linseed oil (ICN)	1.2 ml
	Methylparaben (Sigma)	0.3 g
	Sorbic acid	0.4 g
	Ascorbic acid	2.25 g
	Streptomycin sulfate USP (784 IU/mg)	0.15 g
	10% KOH (to pH 5.5) (Sigma)	8.0 ml
	Sodium ascorbate‡	12.5 ml

* Source: United States Biochemical Corporation, unless otherwise indicated.

† Young poplar leaves collected from field grown hybrid poplars (*Populus* × *euramericana* 'Eugenii'), removed from stems, air dried at 40°C, and ground with a 40 mesh Wiley mill.

‡ Sodium ascorbate solution: 4.2 g sodium carbonate and 9.4 g ascorbic acid dissolved in 100 ml distilled water.

The insect response to dietary trials was measured by placing 5 neonates per 30-ml plastic cup. Each trial was replicated at least two times. Following each trial the recipe with the best insect performance (developmental rate, size, and survival) was used for subsequent trials.

The artificial diet was prepared by transferring the hot, dissolved agar solution (Group A) to a blender. Group B ingredients were then added and blended at low speed. Group C ingredients were added when the mixture cooled to 50-55°C. The mixture was blended for several minutes, dispensed into 30-ml plastic cups or petri dishes, and allowed to cool in a laminar flow sterile hood. The artificial diet was surface sterilized by exposing it overnight to UV light and either used fresh or stored at 4°C until needed.

Adults Maintenance. After adult eclosion, beetles were either maintained on the artificial diet dispensed into 100 × 20-mm petri dishes or transferred directly to fresh foliage in plastic crisper boxes or petri dishes. To maintain foliage-reared adults on the artificial diet, pupae were surface disinfected by soaking them in a 1.0% bleach solution for 2 min followed by two rinses in sterile distilled water. Pupae were kept in a sterile petri dish until adults emerged.

Adult longevity was measured for both leaf beetle species by placing 10 foliage-reared adults per petri dish and observing daily mortality for a maximum of 80 days. Adults were given fresh artificial diet approximately once a week.

Comparison of Foliage - vs. Diet-reared Leaf Beetles. Cottonwood and imported willow leaf beetle larvae were reared in groups of six and eight, respectively, on poplar foliage (*Populus* × euramericana 'Eugenii') in petri dishes (100 × 20 mm) to compare insect performance with that of cohorts reared on artificial diet. They were given fresh foliage every 2 to 3 d. Adults from both diet types were weighed within 24 h after they emerged and were maintained individually in petri dishes on fresh poplar foliage. Response variables measured included daily mortality, stage-specific development time, adult weight, and adult longevity.

Statistics. Student's t-tests were used to determine the significance of differences measured between diet- and foliage-reared leaf beetles. The Chi-square procedure was used to compare differences in survival. Systat™ 4.0 was used for all statistical analyses (Wilkinson 1987).

Results and Discussion

Egg Mass Separation and Sterilization. A 0.75% solution of sodium hydroxide was found to be the most effective for separating the eggs in cottonwood leaf beetle egg masses. Egg hatch was not significantly reduced from that of the controls ($P \leq .05$) by using this method. This concentration of NaOH solution was used to remove egg masses from the leaf surface and to separate egg masses for larval rearings.

The cottonwood leaf beetle eggs were more sensitive than willow leaf beetle eggs to the adverse effects of disinfectants. This may have resulted from exposure to NaOH during the egg mass separation procedure. We determined that cottonwood leaf beetle eggs could be surface sterilized best by agitating them for 1 min in 0.05% bleach. Egg hatch was not significantly affected at this concentration. Willow leaf beetle eggs could tolerate up to 0.5% bleach concentration without significant loss of egg viability.

Incubating surface-disinfected eggs on nutrient agar showed that bacterial and fungal contamination was not entirely eliminated. However, only 6% of the cups (n = 117) containing neonates from surface sterilized eggs became contaminated compared to 90% of the cups with neonates from nonsterilized eggs. *Populus* leaf powder also provided antimicrobial protection to the artificial diet. When the diet was prepared with non-nutritive fiber as a substitute for leaf powder, 54% of the cups (n = 78) were contaminated. For this reason we found it necessary to retain the leaf powder as a dietary ingredient, although it was not required for successful larval development.

Rearing Larvae on the Artificial Diet. Dietary ingredients determined necessary for successful development of larval leaf beetles included dextrose, Sure salt mix 2, and rolled oats. Larvae did not develop beyond the second stage when sucrose, Wesson's salt mix, or other protein sources (casein or wheat germ) were substituted for dextrose, Sure 2 salt mix, and rolled oats. Using oats as a major ingredient in insect diets has not appeared previously in the literature (Singh 1977). Changes in concentrations of individual dietary ingredients, except for dextrose, caused only minor changes in survival to the adult stage. Increasing dextrose concentrations increased larval survival from 30% at 0% dextrose to a maximum of 70% at a dextrose concentration of 25% (n = 40). The pH of the artificial diet was adjusted to 5.5 by adding KOH and sodium ascorbate. This pH is similar to that of the cottonwood leaf beetle midgut (Bauer, unpublished). Larval survival and performance were improved by adding KOH and sodium ascorbate together, when compared to larvae reared without either ingredient, with KOH alone, or with sodium ascorbate alone.

Four consecutive generations of cottonwood leaf beetles and three generations of imported willow leaf beetle were reared as larvae on the artificial diet without a decline in insect performance. Interestingly, larvae will not eat foliage after being fed the artificial diet.

Adult maintenance. Adult longevity of cottonwood and imported willow leaf beetles from larvae fed foliage and then maintained as adults on artificial diet was 42.3 ± 4.3 days (n = 43) and 45.5 ± 2.0 days (n = 103), respectively. Adults must be given fresh artificial diet on a weekly basis because surface sterilization of pupae did not adequately suppress microbial contamination. Attempts to improve pupal surface sterilization with higher concentrations of bleach (> 1%), or by prolonged exposure, resulted in unacceptable pupal mortality.

Adults maintained on artificial diet produced few eggs. Although these eggs were viable, they were usually laid directly on the diet surface where they became dehydrated. Therefore, when eggs were desired, we transferred adults to foliage where they resumed normal oviposition. Unlike larvae, adults readily accepted the foliage despite previous exposure to artificial diet. Use of artificial diet for maintaining adults may be useful during periods when foliage is unavailable.

Performance on Artificial Diet vs. Foliage. Survival of cottonwood leaf beetle larvae to the adult stage when reared on the artificial diet was significantly less than that of larvae reared on foliage (Table 2). Larvae reared on the artificial diet also took longer to develop and weighed less as adults compared to cohorts reared on foliage. Adult longevity when reared individually on foliage in petri dishes was similar for both foliage- and diet-reared larvae. However, this was less than the adult longevity in our laboratory colony where groups of adults are maintained in large plastic boxes (54 ± 5.8 d).

Table 2. Comparative performance (mean ± SE) of cottonwood or imported willow leaf beetles reared on diet vs. poplar foliage.

	Cottonwood leaf beetle		Imported willow leaf beetle	
Response variable	Diet-reared	Foliage-reared	Diet-reared	Foliage-reared
Initial instar I (n)	123	48	54	38
Survival to adult (%)	30.1 †	47.9†	42.6‡	39.5‡
Larval period (days)	15.2 ± 0.2*	10.9 ± 0.3*	13.8 ± 0.5*	11.6 ± 0.4*
Adult fresh weight (mg)	18.88 ± 0.72*	23.38 ± 1.23*	4.67 ± 0.16	4.88 ± 0.27
Adult longevity (days)	27.8 ± 4.2	33.0 ± 3.4	26.9 ± 4.7*	49.7 ± 7.1*

* Diet-reared insects were significantly different from foliage-reared insects at the $P \leq 0.05$ level of significance using Student's t-test.

† $Chi^2 = 4.79$, $P \leq 0.03$.

‡ $Chi^2 = 0.02$, $P \leq 0.90$, n.s.

Survival of imported willow leaf beetle larvae reared on the artificial diet was similar to that of cohorts reared on *Populus* foliage (Table 2). However, diet-reared individuals took significantly longer to develop and lived a shorter time than cohorts reared on foliage. Adult fresh weight was similar for both diet types.

Overall performance and quality of imported willow leaf beetles were better than for cottonwood leaf beetles on the artificial diet. Further changes in dietary ingredients may greatly improve the artificial diet for rearing larval cottonwood leaf beetles. In our larboratory, however, we found that the artificial diet was useful for both rearing larvae and maintaining adults during periods when foliage was limited. This artificial diet may also facilitate research that seeks to understand the nutritional interactions between willow- and poplar-feeding chrysomelids and phenolic glycosides (Tahvanainen et al. 1985; Rowell-Rahier and Pasteels 1982).

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