# Artificial Diets for Third Instar Japanese Beetle (Coleoptera: Scarabaeidae)[1]

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J. Entomol. Sci. 29(4): 585-589 (October 1994)

**ABSTRACT** Of four diets tested, one based on lima beans and casein was selected as the best and easiest for rearing third instars of Japanse beetles, *Popillia japonica* Newman. The diet gave the best survival and heaviest pupae and was previously used for rearing larvae of the New Zealand grass grub, *Costelytra zealandica* (White).

**KEY WORDS** Japanese beetle, *Popillia japonica*, artificial diets, larvae.

The Japanese beetle, *Popillia japonica* Newman, is a major pest of turf, pastures, ornamentals, and fruit crops in the northeastern United States (Tashiro 1987). Milky disease, amber disease, green muscardine disease, nematodes, and coccidia are important biological controls of scarab larvae (Klein 1988). Field collected third-instar scarabs are routinely used by research scientists studying insect pathogens, and these studies would be failitated if the insects could be reared or maintained under as close to aseptic conditions as possible. An artificial diet which can be sterilized prior to use is an ideal food source for such rearing.

Goonewardene et al. (1974) developed a diet for rearing larvae of Japanese beetle. This was based on an acetone-extracted grass-clover powder which is complicated and time-consuming to prepare. We evaluated four artificial diets for field collected third-instar Japanese beetle. All diets have been used previously for rearing phytophagous scarabs.

## Materials and Methods

Diet 1 was previously reported by Wigley and Dhana (1992) for rearing post-neonates of the New Zealand grass grub, *Costelytra zealandica* (White). It was composed of three parts (A, B, and Vitamins) and prepared as follows: Part A, 100 g lima beans, soaked and drained, 900 ml water, 40 g agar, 750 mg sodium proprionate, 10 g casien, 750 mg potassium acetate, and 1.25 g sorbic acid; Part B, 350 mg

[1] Accepted for publication 25 August 1994.
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penicillin, 1 ml 50/50 linseed/wheatgerm oil, 100 g oven dried, autoclaved, peat, 350 mg streptomycin, and 4 ml 10% formaldehyde; and Vitamins, 3 g ascorbic acid, 10 mg niacin, 2.5 mg folic acid, 200 ug biotin, 5 mg riboflavin, 500 mg inositol, 2.5 mg thiamine hydrochloride, 2.5 mg pyridoxine hydrochloride, 30 mg calcium pantothenate, 40 ug vitamin B12, and 500 mg choline chloride. The ingredients in Part A were autoclaved, blended, and allowed to cool to 70° C. The ingredients in Part B, and the Vitamins, were then blended into the cooled mixture. The diet was adjusted to a pH of 4.6 with ascorbic acid.

Diet 2 was used by R. H. Goodwin to rear Australian scarab larvae (R. H. Goodwin, pers. comm.). It is prepared by mixing 2 g of methyl-*p*-hydroxy benzoate with 850 ml of water, adding 20 g of agar and stirring and heating until the agar is dissolved. The following are then added in order, stirring after each addition: 2 g Tween® 80 (Atlas Powder Co., Wilmington, DE), 4 g cholesterol, 50 mg α-tocopherol acetate, 5 g soy oil, 2 g choline chloride, 0.4 g inositol, 20 g wheat germ, 10 g torula yeast, 40 g sucrose, and 10 g Wesson salts. The mixture is allowed to cool to 45° C, and 4 g of ascorbic acid and 1 L of ground vermiculite are stirred in.

Diet 3 was used by Toohey (1977) to rear larvae of the melolonthine scarab, *Phyllophaga anxia* (Le Conte). It was prepared by mixing 2 g of methyl-*p*-hydroxy benzoate with 850 ml of water, adding 25 g of agar and stirring and heating until the agar is dissolved. The following are then added in order, stirring after each addition: 2.5 g linseed oil, 30 g wheat germ, 35 g vitamin-free casein, 35 g sucrose, 10 g Wesson salts, 0.5 g chloesterol, 1 g choline chloride, 0.4 g inositol, 1 g sorbic acid, and 0.1 g α-tocopherol acetate. The mixture is allowed to cool to 45° C, after which 4 g of ascorbic acid, 2 ml of 40% formaldehyde, and 10 ml of a B-vitamin mixture are added. The B-vitamin mixture was prepared by mixing the following in 10 ml of water: 10 mg niacinamide, 10 mg calcium pantothenate, 2.5 mg thiamine hydrochloride, 5 mg riboflavin, 2.5 mg pyridoxine hydrochloride, 2.5 mg folic acid, 0.2 mg biotin, and 0.02 mg vitamin $B_{12}$.

Diet 4 was modified from that of Goonewarden et al. (1974) used for rearing *P. japonica*. We heated 20 g of agar in 1 L of water until the agar dissolved. We then added 160 g of acetone-extracted grass-clover [English rye grass, *Lolium perenne* (L), redtop, *Agrostis alba* (L.), and white Dutch clover, *Trifolium repens* (L.)] powder, 4.3 g of Wesson salts, 1.6 ml of linoleic acid, 1.6 ml of linolenic acid, and 1.6 g of choline chloride. The grass-clover powder was from the same stock used by Goonewardene et al. (1974) and had been stored in the dark at 4.5° C.

All diets were poured into 27 × 17.5 × 3.5 cm metal trays to a depth of about 15 cm and allowed to cool before use. Test larvae were collected as third instars on 15 May 1991 from turf near Ashland, OH. Before use they were held in the field collected soil at 15.5° C for 2 days. Each healthy larva was weighed and placed in a 30-ml Conex$^R$ cream and condiment cup (Illinois Tool Works, Inc., Des Plaines, IL) half-filled with moist (2.5% gravimetric soil moisture) sand which had been sterilized at 105° C for 48 h. About 5 g of diet was added, after which the cup was filled with moist sand, capped, and held at 21° C. Fifty larvae were tested on each diet and with no food. Deaths and pupation were recorded every 10 days. The 10-day interval was selected based on previous

experience in rearing grubs and a desire to minimize the adverse effects of frequent handling. The remaining live larvae were weighed and placed in new moist sand and the diet was renewed. Pupae were weighed and sexed using characters on the terminal ventral abdominal segments (Fleming 1972).

We used the Kruskal-Wallis one-way non-parametric analysis of variance and multiple comparison procedure (Conover 1980) to detect differences for the following parameters; relative growth rate for the first 10 days, time to death of larvae, time to pupation of both sexes, and pupal weight of both sexes. Relative growth rate (hereafter referred to as RGR) was calculated as the increase in liveweight in mg/mg of larva/day

$$RGR = [2 (W_{10}-W_0)]/ [10(W_0+W_{10})]$$

where $W_0$ is the initial larval weight and $W_{10}$ is the larval weight at 10 days (Ridsdill Smith and Roberts 1976). Differences in larval mortality and the sex ratio of resultant pupae were detected with the Chi-square statistic in $2 \times 2$ tables.

## Results

At the start of the test, mean weight of larvae was 285.6 mg (SE 2.8, range 173.0-400.6 mg); there were no significant differences between weights of larvae placed on the various diets (ANOVA, $F = 0.22$, df = 4,245, $P = 0.93$).

Significant differences were found in the RGR of larvae at 10 days on the various diets (KW statistic = 28.40, df = 4, $P < 0.0001$). Larval weight increased (positive RGR) on diets 1 and 4 (not significantly different between 1 and 4, $P > 0.05$), while larvae on diets 2 and 3, and those unfed, lost weight (Table 1).

Significantly ($P < 0.05$) more larvae died (and hence fewer pupated) on diet 2 and in the unfed treatment than did on the other diets; there were no significant differences ($P > 0.05$) in mortality on diets 1, 3, and 4 (Table 1). The time at which larvae died also varied significantly between treatments (KW statistic = 15.88, df = 4, $P = 0.0032$). Those that were not fed died significantly ($P < 0.05$) later than those that died on diets 1, 2, or 4 (Table 1).

There were significant differences among treatments in time to pupation for males (KW statistic = 13.59, df = 4, $P = 0.0087$), but not for females (KW statistics = 6.60, df = 3, $P = 0.086$); the single female pupae from diet 2 was not included in the analyses. Unfed males were the slowest to pupate ($P < 0.05$) (Table 1).

There were significant differences in pupal weight for both males (KW statistic = 15.04, df = 4, $P = 0.0046$) and females (KW statistic = 8.72, df = 3, $P = 0.033$). Males were significantly ($P < 0.05$) heavier on diets 1 and 2 than on diet 3 or where they were unfed; there was no significant ($P > 0.05$) difference in male pupal weights on diets 1 and 4 (Table 1). Females were significantly ($P < 0.05$) heavier on diet 1 than on diet 3, with no significant ($P > 0.05$) differences in female pupal weights on diets 1 and 4 and where they were unfed (Table 1).

There was no significant ($P < 0.05$) departure from 1:1 in the male pupae: female pupae ratio on any diet or where they were unfed except for diet 2 where only one female survived (Table 1).

**Table 1. Growth and mortality parameters of third-instar Japanese beetles placed on artificial diets (n = 50).**

| Parameter* | Diet | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Unfed |
| RGR** of larvae | 1.5 (1.0) a | -2.1 (1.0) b | -6.5 (1.4) c | 2.1 (1.4) a | -9.8 (1.0) d |
| Number larvae dead | 14 c | 42 a | 11 c | 18 c | 29 b |
| Time to death† | 2.9 (0.3) b | 2.9 (0.1) b | 3.5 (0.7) ab | 2.9 (0.3) b | 3.9 (0.2) a |
| Time to pupation† | | | | | |
| – males | 2.2 (0.1) b | 2.0 (0.0) b | 2.4 (0.1) b | 2.2 (0.2) b | 2.8 (0.1) a |
| – females | 2.3 (0.1) a | 2.0‡ | 2.3 (0.1) a | 2.6 (0.2) a | 2.7 (0.1) a |
| Pupal weight (mg) | | | | | |
| – males | 232.5 (4.7) ab | 241.4 (5.7) a | 213.2 (5.9) c | 222.4 (4.7) bc | 210.7 (7.7) c |
| – females | 282.5 (6.8) a | 262.5 ‡ | 251.0 (7.2) b | 271.3 (5.3) ab | 261.9 (9.5) ab |
| Number pupating | | | | | |
| – males | 17 | 7 | 18 | 11 | 10 |
| – females | 19 | 1 | 21 | 21 | 11 |

* Standard errors given in parentheses. Means followed by different letters within a row are significantly different ($P < 0.05$).
** RGR = Relative Growth Rate.
† Given in 10-day periods.
‡ Single female pupa - no standard error.

## Discussion

The differences in RGR over the first 10 days show that Japanese beetle larvae grew best on diets 1 and 4. There appeared to be some feeding on diets 2 and 3 as the RGR was greater than where the larvae were given no food. Time to death of larvae did not differ among any of the four diets. However, it was significantly longer in the unfed larvae than in diets 1, 2, and 4, reflecting that many unfed larvae starved to death, or that a bimodal pattern of survival is being utilized.

Diet 2 is unacceptable for maintenance or growth of Japanese beetle larvae because mortality was even higher on that diet than where the larvae were not fed. This may indicate that some of the components in the concentrations used in diet 2 are toxic to Japanese beetle. Diet 3 is less desirable for rearing larvae because, although it gave similar survival to diets 1 and 4, it resulted in a significantly lower RGR, and male and female pupae weighed less than those on diet 1. Pupal weights on diet 3 were not statistically different than those on diet 4.

Larval development on diets 1 and 4 did not differ significantly from each other in any of the parameters we measured. However, preparation of the acetone-extracted grass-clover powder used in diet 4 is somewhat complicated, whereas all of the diet 1 ingredients are available commercially. Diet 4 also contains linoleic acid and linolenic acid, both of which are expensive and create handling problems because they are classified as irritants. In addition, diet 4 lacks antifungal and antibacterial agents present in diet 1, which means that storage of diet 4 for long periods even under refrigeration is difficult. Although lack of antibiotics may be an advantage in testing microbial agents, we conclude that diet 1 is best for short term rearing third instars of Japanese beetles.

## Acknowledgment

We thank Lisa Slater for her technical assistance. Funds for Allsopp's visit to Wooster were provided by the Australian Sugar Research & Development Corporation.

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