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Effects of different diets on the survival, longevity and growth rate of the Annam stick insect, *Medauroidea extradentata* (Phasmatodea: Phasmatidae)

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Abstract

The effects of different food plants (spinach, carrots, organic and non-organic lettuce, defrosted frozen oak leaves) on the longevity, growth rate and survival of the Annam stick insect *Medauroidea extradentata* (Brunner von Wattenwyl) were studied. Among the food plants tested, organic lettuce and defrosted oak leaves were the best food alternative during winter time for maintaining live cultures of *M. extradentata.*

Key words

Phasmatodea, Phasmatidae, *Medauroidea extradentata*, food plants

Introduction

Medauroidea extradentata (Brunner von Wattenwyl), commonly known as the Annam stick insect, was originally recorded from Annam (Vietnam) and a bisexual culture was first brought to Europe in 1949. Since then, it has become a common laboratory insect, especially in Europe (Carlberg 1981). This wingless species is easy to breed and in many stocks males have died out, leaving parthenogenetic cultures (Brock 1992). Other than Bergerard (1958) and Lau (1987), little detailed research has been done on the biology of *M. extradentata*.

The natural food plant of *M. extradentata* is unknown (Carlberg 1987), but in captivity it is known to accept a variety of plants such as *Rubus* spp., *Rosa* sp., *Sorbus* spp., *Fragaria ananassa* Duch, *Cotoneaster* sp., *Pyracantha coccinea* M. Roemer, *Filipendula* sp. (Rosaceae), *Cornus* sp. (Cornaceae), *Quercus* sp. (Fagaceae), *Eucalyptus* spp. (Myrtaceae), *Betula* sp., *Alnus* sp. (Betulaceae), and *Ribes rubrum* L. (Grossulariaceae), (Carlberg 1987, Lau 1987).

Obtaining sufficient quantities of these food plants in temperate countries during winter time can sometimes be inconvenient. The objectives of our study were to find an alternative, easily accessible, winter food source for *M. extradentata*, and to test the effects of these food sources on its longevity, growth rate and survival.

Material and Methods

The study was conducted from 11 October 2000 to 30 October 2002 at the Lyman Entomological Museum of McGill University, Quebec, Canada. Adult females of *M. extradentata* were originally received from the Insectarium of Montreal. Newly hatched nymphs of *M. extradentata* were placed in glass jars 12.5cm (L) × 12.5cm (W) × 21.5cm (H) covered with a screw-cap with wire mesh in the center. The wire mesh provided an anchor point for molting and allowed air circulation. When nymphs reached about 7cm in length, each was transferred into a glass terrarium 12cm (W) × 45.5cm (L) × 42cm (H) covered with a wire mesh top. Only one stick insect was kept in each bottle or terrarium. The bottom of each cage was lined with paper toweling. A wooden branch extending diagonally between opposite corners was added to each terrarium, as an additional anchor point for molting. Insects were kept at room temperature (22 ± 1°C) with a photoperiod of 12 h.

Five different diets were tested on *M. extradentata*: nonorganic carrot, *Daucus carota* L. (Apiaceae); defrosted frozen leaves of northern red oak, *Quercus rubra* L. (Fagaceae); fresh leaves of spinach, *Spinacia oleracea* L. (Chenopodiaceae); nonorganic Romaine lettuce and organic Romaine lettuce, *Lactuca sativa* L. (Asteraceae). The defrosted oak leaves, organic lettuce and carrots were each tested on 7 females; the nonorganic lettuce treatment was tested on 14 females (the number of individuals tested was higher for this treatment as the experiment was performed twice). The spinach diet was tested on 8 females, although 3 individuals were removed from the experiment after 4 months, when the diet was obviously inappropriate and further continuation of the experiment was unnecessary. In addition to these diets, one female fed on fresh leaves of northern red oak for the first 3.5 months of its life was used for comparison with the other diets (although not included in the statistical analysis).

Carrots, spinach and both organic and nonorganic lettuce were purchased in local grocery stores. Oak leaves were collected from trees on Macdonald Campus of McGill University in St-Anne-de-Bellevue and, for the defrosted leaf treatment, were kept in a plastic bag stored in a freezer for at least one month prior to use. All food was washed with tap water before use. The outer layer of leaves of non-organic lettuce was not used and carrots were peeled and sliced thinly before feeding. Carrots, spinach and lettuce were placed on a ball of wet paper toweling in a plastic Petri dish and were changed daily. Frozen oak leaves were changed every 2nd day and fresh oak every 3rd day. For oak treatments, one medium to large leaf was placed in the cage with its petiole in a small bottle of water closed with a cotton ball. Insects were fed late in the day just before the end of the light cycle. Water was sprayed inside the cage and on the food every day and additional drinking water was provided in small Petri dishes at the bottom of the cage (cages with early nymphal stages were sprayed only lightly).

Seven parameters, defined as follows, were measured:

Longevity (all individuals): number of days from birth to death, recorded for all individuals of each treatment, except those fed with frozen oak (date of death was not recorded for these insects) and 3

individuals on the spinach diet (see above).

Longevity (adults only): number of days from birth to death, recorded for all individuals that reached adult stage. Longevity (adults only) was calculated for each treatment except defrosted oak (date of death was not recorded). An individual was considered an adult once it had laid eggs.

Duration of nymphal stage: number of days from birth to reach maximum length, calculated only for individuals that reached adult stage.

Duration of adult stage: number of days from the time an adult reached maximum length to the time of death.

Growth rate: maximum length of a specimen, minus initial length, divided by number of days required to reach maximum length (calculated from date of the first measurement). Length was measured from the tip of the abdomen to the base of the antenna every 2 weeks using a vernier caliper. Three individuals fed on spinach and 2 individuals fed on frozen oak died too early for growth rate to be calculated.

Adult final size: maximum length of an adult individual.

Survival: Percentage of individuals reaching adult stage

To test effects of the different food sources on longevity (all individuals and adults only), duration of nymphal stage, duration of adult stage, growth rate and adult final length of *M. extradentata* we used a one-factor analysis of variance (ANOVA) with different levels of the main factor "food source" (Spinach, Carrot, Non-organic Lettuce, Organic Lettuce, Defrosted Oak) depending on available data. The Kolmogorov-Smirnov test for normality was used and non-normally distributed data were log-transformed. All tests were performed using SAS version 5.1 (SAS Institute Inc., Cary, NC).

Preliminary observations on egg laying and hatching were also made for each diet.

Results & Discussion

Spinach.—Individuals fed with spinach had a growth rate lower than most other diets (Table 1, Fig. 1). They also had a short lifespan (46.8 ± 19.0 d) and none reached the adult stage. Five of 8 individuals died in less than 100 d. Among these, 2 lived for 81 d and 99 d, while 3 died between 13 and 18 d. The 3 remaining individuals were removed from the experiment when they were between 123 to 131 d old because the diet was obviously inappropriate and continuation of the treatment was unnecessary. These individuals were not included in the calculation of longevity, as their longevity was not known, but were included in the calculation of growth rate. The results obtained with this diet were similar to those of Hsiung and Panagopoulos (1998) for *Eurycantha calcarata*. In both studies the low survival and low growth rate may be due to the presence of 20-hydroxyecdysone in spinach (Grebenok *et al*. 1991). This is an insect molting hormone that can affect insect development by inhibiting feeding behavior (Tanaka *et al*. 1994).

Carrots.—These individuals had a growth rate comparable to those fed with spinach and significantly lower than individuals fed with all other diet (Table 1, Fig. 1). Although only 57.1% of the individuals reached adult stage, most of them lived for a long time. Among the 3 individuals not reaching adult stage, one died after 82 d, while the other 2 lived for 402 and 504 d which was the highest longevity of all insects in the experiment.

Their nymphal life averaged 333 ± 11.7 d, almost 3× as long as that recorded for the organic lettuce and fresh oak leaves diets (Table 1). Although duration of adult stage lasted only 56 ± 7 d, insects fed with carrots lived overall significantly longer (361.4 ± 49.5 d) than those fed other diets (Table 1). Duration of adult stage for individuals fed with carrot was not significantly different than individuals fed with organic lettuce (Table 1), but this duration was quite short compared to 5 to 7 months recorded by Bergerard (1958) for individuals fed with *Rubus* spp. (at 23˚C). Individual *M.*

Table 1. Effects of different diets on survival, longevity, growth and egg laying of *Medauroidea extradentata*. All values are mean ± S.D.; number of individuals is in parentheses. N/A means no individuals reached adult stage. The single individual fed fresh oak leaves was not tested statistically. *Post-hoc* comparison of means was made using the least significant difference (LSD), with significant differences ($P < 0.01$) indicated by different lower case letters.

Food Source	% survival	Longevity (All individuals) (d)	Longevity (Adults only) (d)	Duration of nymphal stage (d)	Duration of adult stage (d)	Growth rate (mm/d)	Adult final size (mm)	Eggs laid	Eggs hatched
Spinach	0 (5)	46.8 ± 19.0 c (5)	N/A	N/A	N/A	0.09 ± 0.02 c (5)	N/A	N/A	N/A
Carrot	57.1 (7)	361.4 ± 49.5 a (7)	389.0 ± 7.77 a (3)	333.0 ± 11.7 c (3)	56.0 ± 7.0 a (3)	0.18 ± 0.03 c (7)	92 ± 1.6 b (3)	Yes	No
Non-organic Lettuce	0 (14)	63.4 ± 9.1 c (14)	N/A	N/A	N/A	0.45 ± 0.05 b (14)	N/A	N/A	N/A
Organic Lettuce	85.7 (7)	172.0 ± 39.7 b (7)	197.4 ± 36.1 b (6)	125.7 ± 2.7 a (6)	71.7 ± 35.1a (6)	0.64 ± 0.09 a (7)	99 ± 1.7a (6)	Yes	Yes
Defrosted Oak	71.4 (7)	No data*	No data*	192.4 ± 8.1 b (5)	No data*	0.40 ± 0.03 b (5)	87 ± 1.96 b (5)	Yes	Yes
Fresh Oak	100 (1)	210 (1)	210 (1)	123 (1)	87 (1)	0.63 (1)	88 (1)	Yes	Yes

* See explanation in text

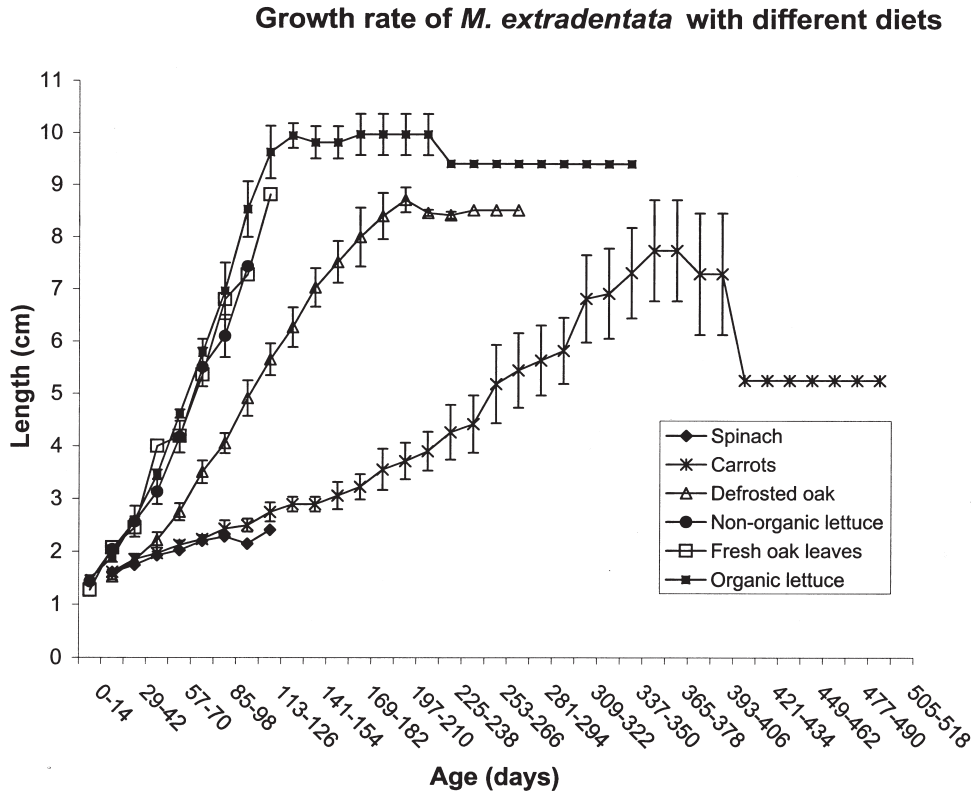

Fig. 1. Growth of individuals of *M. extradentata* fed with spinach, carrot, non-organic lettuce, organic lettuce, defrosted oak leaves and fresh oak leaves diets.

extradentata fed with carrot were significantly smaller than those fed with organic lettuce (Table 1, Fig 2). A few of the adults laid eggs, but these failed to hatch. It seems that the effects of the carrot diet are comparable to the effects of juvenoids: they extend the nymphal stage for an unusual period of time and insects reaching the adult stage are sterile. In our study, this may have been due to pesticide residues present on the carrots.

Non-organic lettuce.— All individuals fed with non-organic lettuce failed to reach the adult stage and died suddenly at a young age. The experiment was performed twice with this diet. In the first experiment, 6 of 8 individuals died on the same day. The insects were between 24 and 73 d old at the time. The 2 remaining individuals lived for 118 to 120 d. The second experiment was performed on 6 individuals. Three of them died in less than 40 d and the remaining individuals lived for no more than 100 d. The longevity calculated for all individuals fed with non-organic lettuce was 63.4 ± 9.1 d. This is comparable to the longevity of individuals fed with spinach (Table 1, Fig. 1). The high mortality was most likely due to insecticide residues present on the lettuce (even though the leaves were rinsed carefully before use). Chemicals had also an effect on growth of the insects, as their growth rate (0.45 ± 0.05 mm/d) was significantly lower than that calculated for individuals fed with organic lettuce (0.64 ± 0.09 mm/d).

Organic lettuce.—Most insects (85.7%) fed with this diet reached adult stage. Their growth rate (0.64 ± 0.09 mm/d) was significantly higher, and the duration of nymphal life (125.7 ± 2.7 d) significantly shorter, than with the other diets (Table 1). The duration of nymphal life was also short compared to 160 to 179 d recorded by Lau (1987) for individuals feeding on multiple host plants (at 18 to 25°C), but similar to individuals fed with *Rubus* spp. (at 23°C), which took 4 to 6 months to reach the adult stage (Bergerard 1958). However, the average duration of the adult stage for the individuals fed with organic lettuce was 71.7 ± 35.1 d (2.3 months), compared to 5 to 7 months recorded by Bergerard (1958). Only one individual lived noticeably longer (361 d) than the others, including 218 d (over 7 months) as an adult. Bergerard (1958) noted that some unfertilized females of *M. extradentata* have a very short adult life, and may die after 2 to 3 months. These females had a partial retention of eggs in their oviducts and unfertilized females that lived longer had empty oviducts when dissected (Bergerard 1958). This may explain the short adult life of the majority of the individuals that were fed with organic lettuce.

The adult body size (99 mm) for individuals fed with organic lettuce was similar to the 95 mm normally recorded for this species (Carlberg 1987) and also exceeded the size recorded for individuals in other treatments (Table 1). Among the individuals reaching adult stage, all laid viable eggs.

The duration of nymphal stage, adult stage and growth rate of individuals fed with organic lettuce were similar to values obtained for the individual fed with fresh oak (Table 1), which suggests that organic lettuce may be a good alternative winter food for *M. extradentata.*

Defrosted oak leaves.—Most individuals (71.4%) fed with frozen oak leaves reached the adult stage. None of the *Eurycantha calcarata* fed defrosted oak by Hsiung and Panagopoulos (1998) reached the adult stage. Given that a culture of *E. calcarata* currently kept at the Lyman Museum is fed during winter with defrosted oak leaves with no apparent negative effects on the immatures or adults, this difference may be due to the fact that the leaves were changed every 3 d in their study, compared to every 2nd d in our study. In our experiment the 2 individuals of *M. extradentata* not reaching adult stage died

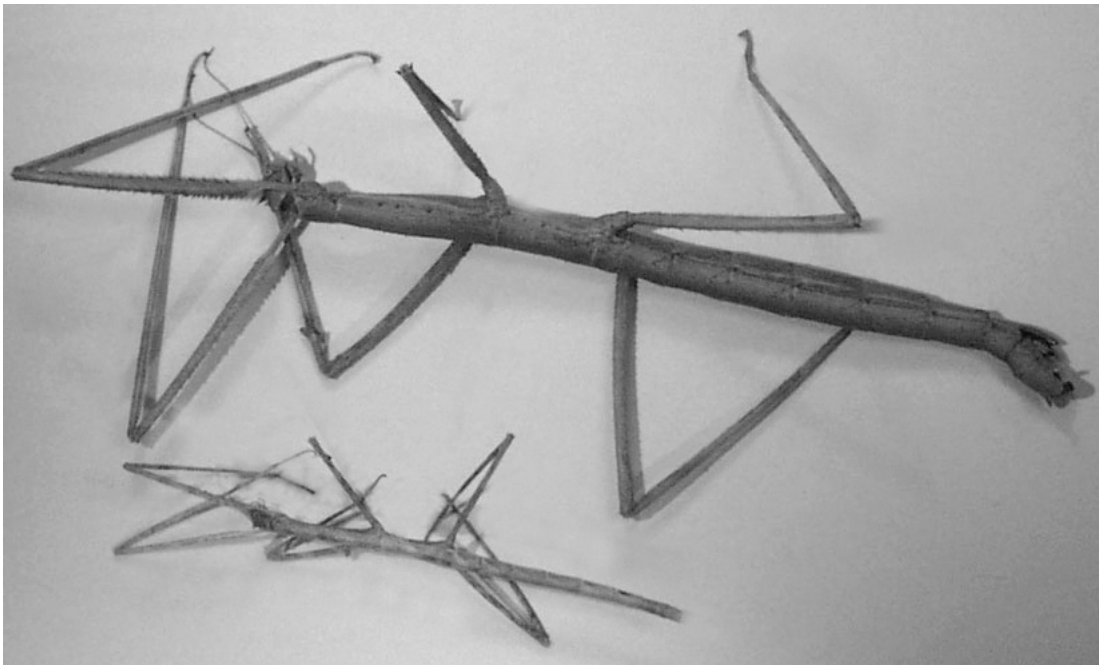

Fig. 2. Size difference between an individual fed on carrot diet after death at 504 d (52.5 mm) and an individual fed on organic lettuce after death at 140 d (101 mm).

at a very early age (13 d and 31 d); this may be due to the fact that the mandibles of those nymphs were not strong enough to pierce the tougher defrosted oak leaves. Growth rate in this treatment was lower than that for the individuals fed with organic lettuce or fresh oak (Table1, Fig 1). Lower growth rate also resulted in a longer nymphal life for this treatment (Table 1). Longevity (all individuals) of insects fed with this diet was not recorded, but the 5 individuals reaching adult stage laid eggs at 203, 204, 217, 232, and 269 d old, which makes a minimum longevity of 222.8 ± 10.3 d (not included in Table 1). This longevity is higher than all other treatments except carrot. The lower growth rate for these individuals compared to the fresh oak treatment may indicate that defrosted oak leaves had a lower nutritive value, although longevity (all individuals) and adult final size of individuals fed with defrosted oak did not support this. Overall, defrosted oak leaves seem to be an acceptable winter food source for *M. extradentata*.

In conclusion, among the 5 food plants tested, organic lettuce was the best alternative food plant during winter. Organic lettuce had the highest survival rate and fastest growth rate and gave the most similar results to fresh oak leaves. Defrosted oak leaves could also be used as an alternative to fresh oak. Although individuals fed with defrosted oak had a slower growth rate, most survived to the adult stage and laid viable eggs. Even though individuals fed with carrots lived for a longer period of time than for all other diets, we would not recommend carrots as an alternative food source. These individuals had an abnormally low growth rate and they did not lay viable eggs. Spinach and non-organic lettuce are definitely not recommended as alternative food plants as the individuals had a short longevity and none reached the adult stage. Further testing should focus on the use of a mixed diet such as a combination of carrots, organic lettuce and defrosted oak leaves. It would also be interesting to test organic carrots to see if this would result in a faster growth rate or increased egg viability than with the non-organic carrots used in this experiment.

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