Rearing the Cannibalistic Larvae of *Calosoma sycophanta* (Coleoptera: Carabidae) in Groups[1]

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ABSTRACT Cannibalistic immatures of the gypsy moth (*Lymantria dispar* L. [Lepidoptera: Lymantriidae]) predator *Calosoma sycophanta* L. (Coleoptera: Carabidae) must usually be reared individually. This paper describes a method of rearing *C. sycophanta* larvae in groups by placing them in plastic containers having 3 cm of moist peat moss in the bottom. Larvae are fed gypsy moth pupae placed on top of the peat moss. Under these conditions, larval mortality is about 50%. By varying the number of prey, it was found that larval mortality increased if less than 0.5 pupae/day/larvae were provided. However, when not starved, at least 40 *C. sycophanta* larvae can be reared per container with no increase in mortality. Larvae were seen to burrow through the peat moss extensively, which may reduce cannibalism. Higher larval mortality occurred when using paper towelling (wet or dry) as the substrate. Group rearing will make it easier to supply beetles for augmentative releases against the gyspy moth.

KEY WORDS *Calosoma sycophanta*, cannibalism, Carabidae, ground beetle, gypsy moth, *Lymantria dispar*, Lymantriidae, predator, rearing method.

Calosoma sycophanta L. (Coleoptera: Carabidae) is an introduced, established predator of the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), in the United States. Adults may live for 3 to 4 years (Burgess 1911). In southern New England, the beetle lays eggs in soil only if abundant lepidopterous larvae are present during June (Weseloh 1985a, 1985b, 1993). Adults enter the soil in late June and become dormant until the next year. The immature stages develop very rapidly in late June and early July, feeding primarily on exposed lepidopterous pupae on tree trunks. After about 10 days the last larval stage enters the ground and pupates. Adults develop by August, but remain in the soil until the next year. Thus, *C. sycophanta* is only active for a short period of time each year. In southern New England, its phenology and behavior means that it feeds almost exclusively on the gypsy moth, the only prey species that commonly has large populations in late spring.

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There is evidence that *C. sycophanta* can be an effective natural enemy. Releases in Connecticut have caused transitory increases in gypsy moth mortality (Weseloh 1990). The beetle readily reproduced the same summer it was released in areas in Michigan and Delaware where it had not previously been present (Weseloh et al., unpublished). Because of the high reproductive potential of *C. sycophanta*, it appears that about 400 adult beetles per ha are enough to decrease prey populations to innocuous levels (Weseloh 1985a, 1985b).

Rearing *C. sycophanta* is difficult because its larvae are cannibalistic (personal observations). Because of its potential as a natural enemy, particularly as an augmentative release agent, I developed a procedure for rearing *C. sycophanta* immatures in groups. This paper describes the method and the effect of experimental manipulations on rearing success.

Materials and Methods

Description of Final Rearing Method. The colony of *C. sycophanta* used in the experiments was originally started from adults collected in Connecticut forests in the early 1980's. Gypsy moth larvae (for *C. sycophanta* adults) and gypsy moth pupae (for beetle larvae) obtained from USDA-APHIS (Methods Development Laboratory, Otis ANGB, MA) were used exclusively for rearing the predator. Adult beetles were stored at 4°C individually in 30-ml transparent plastic creamer cups (capped) which were half-filled with moist peat moss. They can be stored for more than a year with little mortality (<10%). All rearing occurred in a room kept at approximately 25°C, 50% RH and 14 h light; 10 h dark per day. After removal from storage for rearing, mating pairs were placed in 15 × 3 cm plastic Petri dishes, the bottom of which had a 9-cm diam piece of Whatman® #3 filter paper and 3.5 × 1 diam cm glass vial containing rolled, moist paper towel. Each pair was provided two to eight gypsy moth larvae (instars four to seven) daily. When a female began laying eggs, the pair was placed in a 9 cm high × 9 cm diam glass jar that was 1/4-filled with moist peat moss. The top was closed with a screen held in place by a plastic screw-cap having a 7-cm diam hole cut in it. Gypsy moth larvae were supplied as before. Females readily laid eggs in the peat moss, especially at the bottom of the container. The oblong, white eggs (1 × 2 mm) were removed daily and placed in 10 × 1.5 cm plastic Petri dishes lined with water-saturated filter paper and held until hatch. The peat moss was replaced when eggs were harvested.

Because adults are not cannibalistic, an alternate, less labor-intensive method of obtaining eggs also was used. In this method, up to 10 pairs of adults were placed in a 32 × 21 × 10 cm clear plastic container with a screen top and the bottom lined with paper towels. A 6 × 1.5 cm plastic Petri dish bottom containing wet cotton was placed in each container, as was a 15 × 3 cm Petri dish bottom filled with moist peat moss. About 30-40 5th and 6th instar gypsy moths were also provided. After 1 to 2 weeks, females began laying eggs in the peat moss. Food and paper towelling were changed daily, and the container of peat moss was changed when eggs were harvested every few days.

In the laboratory, females may lay hundreds of eggs over a period of up to 2 months (Burgess 1911). Beetles that no longer reproduced were placed individually in 30-ml creamer cups half-filled with moist peat moss and held for about

1 month at room temperature. They were then placed in a dark, 4°C temperature room. After approximately 4 months in cold storage, these beetles can be used to produce eggs again. Each beetle may have several cycles of reproduction during its lifetime (Burgess 1911).

When first instars hatched, they were placed in groups (numbers given below) in 32 × 21 × 10 cm plastic containers that had 3 cm layers of moist peat moss in the bottom. Gypsy moth pupae (numbers given below) were placed on top of the peat moss, and a screen lid covered the top. Additional pupae were provided periodically (see below), but no pupae were removed and the peat moss was not kept moist. The peat moss absorbed liquids that leaked from the prey as *Calosoma* larvae ate. Larvae burrowed through the peat moss, and this presumably enabled them to avoid encounters with other larvae that could lead to cannibalism. After larvae had completed development, they were retrieved by sifting the substrate through a hardware cloth grid having 0.6 cm holes. Each larva was placed individually in a 30-ml creamer cup half-filled with moist peat moss that was held at room temperature until pupation and adult emergence occurred (1-2 months). Adults were then stored at 4°C. Newly-emerged adults were not fed before being put in cold-storage. Attempts had been made to hold pupating larvae in groups, but excessive mortality made it necessary to handle these individually.

Experimental Manipulations of Rearing Conditions. To explore aspects of rearing that are important for larval development, several experiments were conducted. In the first of these, 18 to 25 first instars were placed in each of 9 containers as described above and fed an excess of gypsy moth pupae (typically 40 to 50 pupae every 3 days). Numbers of surviving larvae were counted daily until all had completed development.

The effect of limited prey numbers was investigated in a second experiment. Twenty *C. sycophanta* first instars were placed in each of 30 containers that were divided into 3 groups of 10 each. Every third day while larvae were developing, 10, 20 or 30 fresh gypsy moth pupae were placed in containers in each group. At the end of larval development (usually about 2 weeks), the numbers of surviving *C. sycophanta* were counted.

To determine the effect of crowding on survival of *C. sycophanta* larvae, 25 containers with moist peat moss and excess numbers of gypsy moth pupae were set up as above. Either 20 (15 containers) or 40 (10 containers) *C. sycophanta* first instars were placed in each container and reared with an excess of prey until larvae stopped eating. The numbers of surviving *C. sycophanta* in each container were determined.

The last experiment involved comparing paper towelling and peat moss as substrates. Unless otherwise indicated, 20 first instars were placed in each container, and gypsy moth pupae were always supplied in excess. The numbers of larvae surviving in each container were determined at the end of larval development. Substrate types were tested in pairs as follows: (1) Dry paper towelling vs paper towelling water-saturated for the first 3 days of the test. (2) Dry paper towelling vs 3 cm of moist peat moss. (3) Water-saturated paper towelling vs 3 cm of moist peat moss. (4) 1.5 cm of moist peat moss vs 3 cm of moist peat moss. In test number (4), 18 to 50 larvae were used per container. The number of replicates for each experiment are given with the results.

Data were analyzed by one-way analysis of variance and means were compared with treatment contrasts using the MGLH module of SYSTAT (Wilkinson 1988), or by two-tailed t-tests using the STATS module of SYSTAT.

Results

The average daily survival for larvae reared in nine containers with peat moss, when excess prey were provided, are given in Fig. 1. Mortality occurred both early and late in larval development and, judging by the slope of the survival curve, tended to be somewhat higher for young larvae. However, substantial mortality occurred for older larvae as well. Cumulative mortality rates were higher than 50%.

Limiting the number of prey decreased *C. sycophanta* larval survival. Only 5.3 ± 1.7 (standard deviation) out of 20 larvae given 10 pupae survived, while 8.8 ± 2.7 given 20 pupae, and 12.8 ± 2.4 given 30 pupae survived. All means were significantly different from each other by one-way analysis of variance ($F = 23.696$; df = 2, 27; $P < 0.001$) and compared treatment contrasts ($P < 0.05$). At 30 prey per container, about half the larvae survived. This appears to be the maximal survival rate when prey are unlimited (Fig. 1). Assuming then that 30 caterpillars supplied every three days is adequate for 20 beetle larvae, it appears that a feeding rate of 30 pupae/3 days /20 larvae = 0.5 gypsy moth pupae/day/larva prevents starvation.

Crowding of *C. sycophanta* larvae did not affect survival rates. At 20 larvae per container, 66.3% ± 16.1 (standard deviation) survived, and at 40 larvae, 60.0% ± 16.5 survived. These differences are not significantly different by two-tailed t-test ($t = 0.955$, df = 23, $P = 0.350$). Thus, more than 50% of larvae survived whatever the crowding regime, and at least 40 larvae can be reared per container without undo adverse effects of crowding.

Results of tests using different substrates are given in Table 1. Wet paper towelling supported better survival than did dry towelling. This is probably because neonate *C. sycophanta* often do not start feeding for 1 to 2 days after hatch. The moisture apparently prevents them from drying out. Dry towelling was much less, and wet towelling somewhat less effective than peat moss. This difference is probably because of cannibalism, as the towels probably do not provide adequate places for larvae to hide. However, there was no significant difference between the survival of larvae placed in 1.5 cm vs 3 cm of peat moss. Presumably, larvae were able to avoid each other even when the amount of peat moss was halved.

Discussion

In none of these tests did survival of *Calosoma* larvae greatly exceed 50%. This is considerably less than when larvae are reared individually (survival often above 70%, personal observations). Visual inspection of the survival curve of Fig. 1 shows that young larvae had lower survival rates than did older larvae, even though large instars consume much more prey (Weseloh 1988). Thus, food supply was probably adequate. Cannibalism might still be a factor, but mortality was essentially equal at high and low larval densities. The reason for the excess group mortality is unknown.

Table 1. Effect of substrate on survival of *Calosoma sycophanta* larvae.

Substrate	Number Replicates	Percent Surviving ± standard deviation	*P* value*
Dry towelling	20	20.5 ± 7.2	0.015
Wet towelling	20	29.5 ± 2.83	
Dry towelling	10	21.0 ± 11.8	0.002
3 cm peat moss	10	51.0 ± 23.2	
Wet towelling	21	38.6 ± 19.4	0.051
3 cm peat moss	20	50.8 ± 19.3	
1.5 cm peat moss	11	39.9 ± 39.7	0.358
3 cm peat moss	10	47.9 ± 13.9	

* Two-tailed t-test used to determine differences between pairs of averages using the STATS module of SYSTAT (Wilkinson 1988).

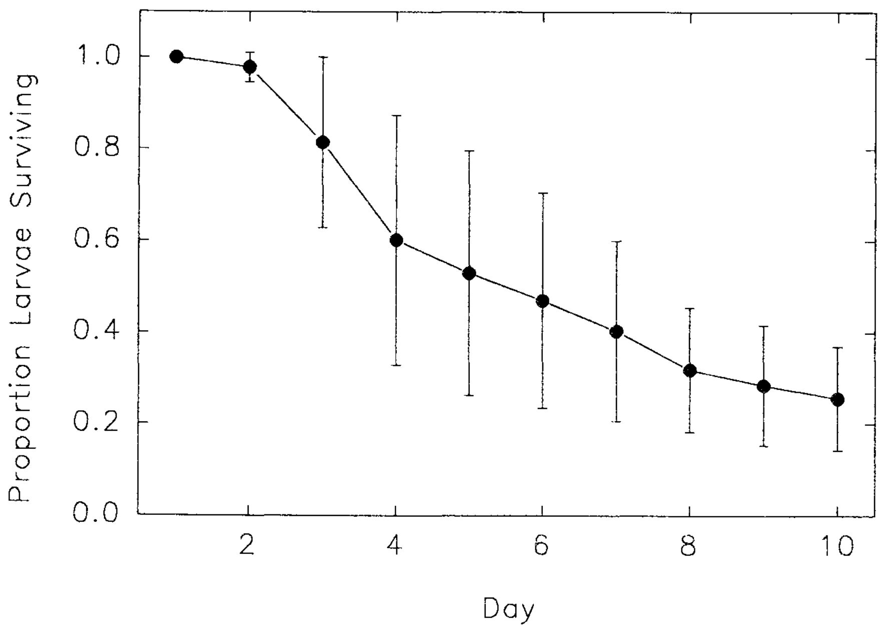

Fig. 1. Daily average survival rates (± standard deviations) from hatch to day 10 of *Calosoma sycophanta* larvae in 9 containers provided with excess gypsy moth pupae.

Despite the undocumented mortality, group rearing of *C. sycophanta* larvae is still cost-effective. Assume conservatively that 100% of larvae survive when reared individually, and that 50% survive when reared in groups of 40. Then 20 larvae can be reared together using the same effort needed to rear one individually. The costs of harvesting eggs and preparing larvae for pupation are the same for both methods, but the labor-intensive and repetitive process of feeding larvae is very much diminished when rearing is carried out in groups. Once larvae have completed their development, they are a valuable resource that can be handled individually. The procedure outlined here is an improvement over individual rearings, and could easily be scaled up. An efficient procedure for rearing beetles will facilitate biological control programs that stress augmentative releases.

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