Turkish Journal of Zoology

Volume 30 | Number 2 Article 10

1-1-2006

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Turk J Zool
30 (2006) 181-185

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Received: 19.07.2005

Abstract: This study was conducted to determine the mass production of Calosoma sycophanta L. under laboratory conditions (23 °C, 60%-65% RH and a photoperiod of 8:16 (L:D) h, 85%-90% soil humidity) between 2001 and 2004 in Kahramanmaraş. The adult emergence period of C. sycophanta started on 21 February and extended until 7 March (from soil). When they emerged, they fed on caterpillars of the pine processionary moth. The egg laying period continued for 20-25 days with a hatching period 6-13 days, Three larval instars were observed. Duration of the first instars was 7-11 days, of the second was 8-12 days, and of the third was 15-18 days. The pupal stage of the beetle continued for 9 to 16 days. During this application pupae should be put into humid soil 25-30 cm deep. Approximately 200-250 laboratory reared individuals were released per hectare.

Key Words: Calosoma sycophanta, Predator, Thaumetopoea pityocampa, mass production, Kahramanmaraş

Çam Keseböceğine Karşı Biyolojik Mücadelede Kullanılan *Calosoma sycophanta* L.'nın Kitle Üretimi ve Araziye Salımı

Özet: Bu çalışma Calosoma sycophanta L.'nın kitle üretimi amacıyla laboratuar koşullarında (23 °C, %60-65 nem, fotoperiyod 8:16 saat (gece: gündüz), %85-90 toprak nemi) 2001-2004 yılları arasında Kahramanmaraş bölgesinde yürütülmüştür. C. sycophanta erginlerinin bölgede topraktan çıkışları 21 Şubat -7 Mart tarihleri arasındadır. Erginler topraktan çıktıklarında çam keseböceği larvaları ile beslenmektedirler. Yumurta bırakma periyodu 20-25 gün, açılma süresi ise 6-13 gün sürmektedir. C. sycophanta üç larva dönemi geçirmekte, birinci larva dönemi 7-11 gün, ikinci larva dönemi 8-12 gün üçüncü larva dönemi 15-18 gün pupa dönemi ise 9-16 gün sürmektedir. Üretimi yapılan bireyler pupa döneminde 25-30 cm derinliğindeki nemli toprağa hektara 200-250 birey bırakılmaktadır.

Anahtar Sözcükler: Calosoma sycophanta, Predator, Thaumetopoea pityocampa, Kitle Üretimi, Kahramanmaraş

Introduction

The ground beetle, *Calosoma sycophanta* L. (Coleoptera: Carabidae), was exported from Europe to North America to use as a natural enemy against the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) (Weseloh et al., 1995; Schafer et al., 1999). The beetle is well established in the United States to control the gypsy moth. There are several studies showing that the beetle may be a useful natural enemy against gypsy moth outbreaks (Bess, 1961; Campbell, 1967; Weseloh, 1985; Weseloh et al., 1995). The beetle can also feed on different species of Lepidoptera, particularly the pine processionary moth (PPM), *Thaumetopoea pityocampa* (Schiff.).

The ground beetle has been observed during feeding on the PPM, which is a very important pest of pine trees. The PPM feeds on the needles of pine trees, causing annual growth loss, and even the death of trees in some cases. Extensive damage by this pest occurs generally on Calabrian pine trees in different parts of Turkey every year.

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PPM is known to feed on leaves and build large silk nests on the top of the trees. In addition, PPM has urticating hairs, which cause allergies resulting in conjunctivitis, respiratory congestions and asthma (Ziprkowski and Roland, 1966). The PPM is one of the most harmful insect that defoliates *Pinus* (i.e. *Pinus brutia, P. nigra, P. sylvestris, P. pinea,* and *P. halepensis*) as well as species *Cedrus libani* growing in Turkey, and has serious economic importance (Rive, 1966; Lightle and Weiss, 1974; Buxton, 1983; Markalas, 1985; Markalas, 1986; Austra et al., 1987; Markalas, 1989; Avtzis, 1998; Çanakçıoğlu and Mol, 1998; Lyytikainen-saarenmaa, 1999; Tiberi et al., 1999; Avtzis, 2001; Babur, 2002; Kanat et al., 2002). In mature forests, trees are hardly ever killed, but significant losses occur in volume and radial growth (Laurent-Hervouet, 1986). The PPM occurs in regions from sea level to an altitude of 1800 m (Avtzis, 1998; Çanakçıoğlu and Mol, 1998) at different levels of population density in Turkey.

The damage caused by PPM has been mostly observed on *P. brutia* in Turkey, which compose the majority of forests (with more than 3×10^6 ha) and are distributed particularly in the coastal regions of Turkey, namely the Mediterranean (47%), Marmara (40%) and Aegean regions (10%) (SPO, 1995; Çanakçıoğlu and Mol, 1998). It has a volume of more than 1.61×10^8 m^3 and an annual volume increment of more than 5×10^6 m^3, 4×10^6 m^3 of which is allowed to be cut (Bektaş et al., 2003). The PPM created severe damage in an area of about 1.5×10^6 ha in Turkey (SPO, 1995). It was reported that the damage resulting from larvae being nourished with the needles of *P. brutia* and *P. nigra* continued for about 7 or 8 months to a great extent. The larvae at the 4th and 5th instars consume all the leaves of the Calabrian pine trees. Thereby, the damaged trees look like they have been burned (Kanat and Alma, 2004).

It is well known that defoliation may increase tree stress and their susceptibility to secondary pests such as bark beetles and pine weevils, particularly in young trees (Çanakçıoğlu, 1993; Selmi, 1998; Kanat et al., 2002). Outbreaks of PPM are quite periodic, occurring every 5-7 years. It was also reported that losses in total shoots of *P. pinaster* were 41%-50% in moderately defoliated trees and 54%-64% in completely defoliated trees (Markalas, 1998). Due to PPM attacks on *P. brutia* (1-4 m in height) in Turkey, a huge loss of 68% was determined by Babur (2002). Due to PPM damage, the decrease in the annual diameter increment of the Turkish Calabrian pine was on average 21% (Kanat et al., 2005)

Several control methods (mechanical, chemical and biological) have been used against this pest in Turkey but the problem has not been solved completely. In addition, it was observed that a single control method was insufficient to suppress the population outbreak of the PPM. However, biological control is probably the best solution among the different methods to control PPM population outbreaks.

Numerous predators of the PPM have been reported previously (i.e. *Calosoma sycophanta* L. (Coleoptera, Carabidae), *Dermestes undulatus* Brahm. (Coleoptera, Dermestidae), *Formica rufa* L. (Hymenoptera, Formicidae), and *Cuculus canorus* L. (Aves, Cuculidae)) (Oğurlu, 2000).

One of the predatory insects of this pest is *C. sycophanta*, which has been observed to feed on the PPM extensively in Calabrian pine forests (Tosun, 1977; Ogurlu, 2000; Kanat, 2002). This predator consumes both the larvae and pupae of the PPM. *C. sycophanta* as a predator may control the population growth and density of the PPM in outbreaks.

C. sycophanta feeds on *Dasychira pudibunda* (L.) (Lep., Lymantriidae), *Euproctis chrysorrhoea* (L.) (Lep., Lymantriidae), *Hyphantria cunea* (Drury) (Lep., Arctiidae), *Lymantria dispar* (L.) (Lep., Lymantriidae), *Lymantria monacha* (L.) (Lep., Lymantriidae), *T. pityocampa* (Lep., Thaumetopoeidae), *T. solitaria* (Frey.) (Lep., Thaumetopoeidae), and *Tortrix viridana* (L.) (Lep., Tortricidae) (Çanakçıoğlu, 1993; Çanakçıoğlu, 1995; Oğurlu, 2000).

The objectives of this study were to investigate mass production and release of *C. sycophanta* L. to use against the pine processionary moth, *T. pityocampa* (Schiff.), in biological control.

Materials and Methods

This study was conducted under laboratory conditions between 2001 and 2004 in Kahramanmaraş, in the east Mediterranean region of Turkey. The larvae of PPM and the adults of *C. sycophanta* used in this study were collected from different patches of Calabrian pine forests distributed throughout the Kahramanmaraş region and brought into the laboratory.

Adults and larvae of *C. sycophanta* were fed larvae of *T. pityocampa, T. solitaria,* and *Ephestia kuehniella* and liver in the laboratory. Before use, the livers were kept in the refrigerator until they became frozen. Then they were grated to the size that the adults of *C. sycophanta* could eat.

During this study, all research materials were kept under constant conditions, namely 23 °C, 60%-65% RH and a photoperiod of 8:16 (L:D) h, 85%-90% soil humidity. Adults of *C. sycophanta* were collected in March every year. Twenty adults were placed in a plastic crisper (14 x 32 x 12 cm) with 1 PPM silky nest. Predatory beetle collection was performed particularly in Kapıçam, Pınarbaşı and Hartlap near Kahramanmaraş.

The beetles brought from the fields were placed in rectangular (14 x 22 x 8 cm) or round plastic boxes (17 x 15 cm and 19 x 13 cm) in the laboratory. Before placement of the beetles, partly sterilized soil (at 100 °C for 10 h in an autoclave) was put into the boxes (height 3-5 cm).

Deposited eggs of *C. sycophanta* were collected daily. The eggs and newly hatched larvae of *C. sycophanta* were placed in plastic cups (5 x 3 cm). Several holes (5-6) were made in the lid of each cup to provide air. To maintain the humidity of the eggs, they were covered with soil.

Every day newly hatched *C. sycophanta* larvae were transferred into plastic boxes containing moist soil. One larva of *C. sycophanta* was placed in each plastic box to prevent cannibalism among the larvae. They were kept in these boxes until they reached a certain size, 1.2-2.0 cm body length. PPM larvae were provided for feeding every day depending on the consumption capacities of the beetle larvae.

Laboratory reared pupae (200-250) of *C. sycophanta* were released into 1 ha, in which the PPM population was high. The pupae were released into 25-30 cm deep canals in the soil.

Results

The adult emergence of *C. sycophanta* from the soil started in the last week of February and continued until the first week of March. The weight of adults ranged between 0.8 and 1.13 g. The sex ratio of female *C. sycophanta* brought into the laboratory from the field was 0.5. Newly emerged adults from overwintering sites had to feed well on the fourth and fifth instars or pupae of the PPM before mating and oviposition for 1 to 1.5 weeks. This feeding activity is well synchronized with the life stages of the PPM. It occurs when the larvae and early pupae of the PPM are abundant. After this feeding, female adults laid their eggs into humid soil.

The larvae of *Ephestia kuehniella*, given as food to *C. sycophanta*, were very small. Therefore, they can escape from the feeding boxes. Neither larvae nor adults of *C. sycophanta* were able to feed on *E. kuehniella.* The liver stuck to the legs of *C. sycophanta* adults, which prevented their movement. It also adhered to the body of larvae, which resulted in their death. The larvae of *T. solitaria* were suitable for feeding because of their good size and being non-allergic. The larvae of *T. pityocampa* were also good for laboratory feeding of *C. sycophanta*, but they caused an extreme allergic reaction in humans. Therefore, protective measures should be taken before using PPM.

The egg laying period continued until the middle of April, depending on the quality of feeding. If they were not able to find humid soil then they left the eggs on the surface of dried soil. If the humidity was not optimum then the eggs were crystallized. For egg deposition, humid soil is necessary; otherwise eggs lose their viability in dry or wet soil conditions. The rate of hatching larva from eggs is about 85%.

The egg laying period continued for 20-25 days and the hatching period for 6-13 days. Three larval instars were observed. The duration of the first instars was 7-11 days, of the second was 8-12 days, and of the third was 15-18 days. The pupal stage of the beetle lasted 9 to 16 days (Kanat et al., 2005).

When the first instars were passing through the next larval stage, they were yellowish white; they turned brown after 20-25 min and were black over the following 25-30 min.

When the instars reached an average of 20-25 mm in length, they shed their second larval skin. At the end of the last larval stage, the larvae did not feed very much and became virtually inactive. This inactive period continued for 1 week; then they shed their third larval skin and pupated.

After 3 larval stages, *C. sycophanta* became a pupa in the soil. The body length of pupae ranged between 19 and 24 mm.

Callow adults opened the pupal skin from the dorsal side. The length of newly emerged adults was about 2 cm. The antenna, eyes and legs were black. The other parts of the body were yellowish brown.

Some adults emerged with defective body parts from the pupal stage because of either deficit or excessive soil humidity. The pupae reared in the laboratory were released into outbreak regions of the PPM. The number of pupae released in a hectare was between 200 and 250. Pupae were released into ditches (25-30 cm depth) one by one.

The adults of *C. sycophanta* consumed an average of 7 larvae of the PPM daily and they were active 30-40 days a year. One adult of *C. sycophanta* can consume 210-280 larvae of the PPM yearly. When they live 3-4 years then the number of consumed larvae is 840-1120.

The adults of *C. sycophanta* may survive almost 2 months without feeding. In field conditions, the number of larvae of PPM in a silken case was 121 (69-173) and 32 (21-43) in the absence and presence of *C. sycophanta*, respectively. According to these numbers, the population density of the PPM decreased almost 73% because of *C. sycophanta*. The number of adult *C. sycophanta* found in the cases was usually 1 and sometimes 2-3 or more.

Discussion

The biology of *C. sycophanta* is well adapted to the biology of the PPM in the region, which may increase the efficiency of *C. sycophanta* in the event of outbreaks. Similar synchrony has been reported between gypsy moths and the beetles (Weseloh et al., 1995). The beetles are capable of living for 3 or more years and they emerge from the soil when gypsy moth larvae are present in the environment. In the current study, beetles emerged from the soil when PPM larvae were fourth instar. Weseloh (1993) reported that if female beetles did not feed extensively on caterpillars of the gypsy moth within 1 week of emergence, they entered a reproductive diapause even if they obtained prey later. According to the results of this study, females have to feed on caterpillars of the PPM for 1-1.5 weeks before egg deposition. Otherwise, they enter a diapause without laying eggs. Therefore, an abundance of PPM larvae was crucial when *C. sycophanta* emerged from the soil. When the PPM enter the soil for pupation beetles also re-enter the soil. Similar results were reported by Weseloh (1993) for gypsy moths.

Weseloh et al. (1995) suggested that physiology, behavior and phenology make *C. sycophanta* a specific predator and so they can affect lepidopteran species that have life history traits similar to those of the gypsy moth. It was observed that the beetles and the PPM have similar life history traits, presenting a very important opportunity to use this beetle against the PPM. Therefore, we think that this carabid is one of the most important mortality agents of the larvae and early pupae of the PPM. Thus, it should be used intensively in the biological control of the PPM.

Studies have been conducted to establish colonies of beetles and have been augmented through laboratory rearing techniques for field releases to control PPM epidemics in the Kapıçam, Turkoglu, and Suçatı regions (Kahramanmaraş). The effectiveness of the beetles has been evaluated in outbreak populations of the PPM under field conditions.

Mass production and release of *C. sycophanta* continued in Kahramanmaraş, Osmaniye, Balıkesir, Büyükada (İstanbul) in 2004. In addition, mass production was undertaken in Mersin, Adana, Muğla, İzmir, and Çanakkale Forestry Directorate in 2005. In every laboratory, the number of reared *C. sycophantha* was about 10,000. In these regions approximately 200-250 laboratory reared individuals were released per hectare.

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