KFRI Research Report 129

EVALUATION OF PARASITOIDES FOR BIOLOGICAL CONTROL OF THE TEAK DEFOLIATOR

V.V. Sudheendrakumar

KERALA FOREST RESEARCH INSTITUTE
PEECHI, THRISSUR

July 1997 Pages: 32

CONTENTS

ABSTRACT

The biology, behaviour and mass multiplication of two species of indigenous parasitoids of the teak defoliator, **Hyblaea puera** Cramer (Lepidoptera : Hyblaeidae) namely, **Sympiesis hyblaeae** Surekha (Hymenoptera : Eulophidae) and **Palexorista solennis** (Walker) (Diptera : Tachinidae) were studied and the usefulness of these parasitoids as candidates for the biological control programme was evaluated based on their biological characteristics. The acceptance of **H. puera** eggs by two species of exotic egg parasitoids namely **Trichogramma dendrolimi** Matsumara and **T. embryophagum** (Hartig) was also investigated.

S. hyblaeae preferred to lay eggs on first or second instar host larvae. The larvae selected for oviposition was paralysed and a single egg was deposited on the lateral side of the body in the intersegmental region. A single parasitoid laid an average of 15 eggs during its lifespan. Continuous multiplication of the larvae in the laboratory was not possible as the parasitoid entered diapause in the pupal stage during the months February-May. As the diapause period coincides with the early phase of pest build- up, the parasitoid population is not able to numerically respond to the increasing pest population. The scope for mass multiplying the parasitoid for release in the field during the critical period of pest incidence thus appears to be limited. It is concluded that **S. hyblaeae** is not a suitable candidate for use in the biological control programme against the teak defoliator.

P. solennis is an endoparasite and infested third and fourth instar *larvae* of **H. puera.** The female laid an average of 43 eggs during its lifespan. Host larva was not paralysed prior to oviposition. Normally a single parasitoid larva developed within an host. Laboratory studies established the feasibility of continuous rearing of the parasitoid on host larvae. An agar based artificial diet was developed and method of rearing **P. solennis** tested. The feasibility of mass multiplication suggests that this species can be produced in large numbers. However, further refinement of the methods of multiplication is needed before using this species for practical biological control.

Under laboratory conditions the egg parasitoids. **T. dendrolimi** and **T. embryophagum** parasitised **H. puera** eggs. In terms of percentage parasitism **T. embryophagum** was found superior to **T. dendrolimi** Both the species preferred to parasitise fresh eggs and percentage parasitism on one-day old eggs was significantly lower than that on fresh eggs. This indicated that successful parasitism by these parasitoids is dependent on the availability of fresh eggs for their oviposition. It is concluded that the success of using these egg parasitoids in the practical biological control programme depends on our ability to predict the pest incidence and timing of their inundative release in the field.

1. INTRODUCTION

The teak defoliator, *Hyblaea puera Cramer (Lepidoptera: hyblaeidae)* is the most economically important pest of teak in India. It is estimated that defoliation caused by this pest in teak plantation results in significant loss of volumeincrement (Beeson, 1941 : Nair et al., 1985). The biology and ecology this pest has been reported in detail (Beeson, 1941). Recent studies were mostly centered around the population dynamics of the pest and also on its natural mortality factors and control prospects. (Nair *et al,* 1985 : Nair *et al.* 1994).

More than 30 species of parasitoids are known to be natural enemies of *H. puera* (Chatterjee and Misra, 1974). In a more recent study Sudheendrakumar (1986; 1990) reported four species of larval parasitoids namely, *Palexorista solennis* (Walker) (Diptera: Tachinidae), *Sympiesis* sp., (Hymenoptera: Eulophidae) , *Eriborus gardeneri* (Cushman) (Hymenoptera: Ichneumonidae) and *Stictopisthus* sp. (Hymenoptera: Ichneumonidae) from Nilambur. The *Sympiesis* sp. was later identified as *Sympiesis hyblaeae* Surekha (Surekha *et al.* 1995). The above study carried out in Nilambur revealed that the percentage parasitism caused by the indigenous parasitoids was very low and not effective in reducing the pest population. Inundative release of mass multiplied indigenous parasitoids was thus considered to be an ideal pest control option. The present study was undertaken to look into the feasibility of mass multiplication of some selected indigenous parasitoids and assess their efficacy as biocontrol agents.

THE MAJOR OBJECTIVES WERE

1. To study the biology and behaviour of selected indigenous parasitoids,
2. To assess the feasibility of mass rearing of the parasitoids under laboratory condition.
3. To assess the usefulness of the parasitoids for biological control programme based on their biological and behavioural characteristics, and
4. To screen appropriate exotic broad spectrum egg parasitoids of *Trichogramma* spp. against *H. puera.*

2. MATERIALS AND METHODS

General methods are discussed and specifics are given separately under each experiments.

2.1. LOCATION OF THE STUDY

The study was carried out in the laboratories of KFRI at Nilambur and Peechi.

2.2. SELECTION OF PARASITOIDS

2.2.1. Indigenous Parasitoids

Of the indigenous parasitoids reported from Nilambur, the following two species were selected for the study as they were the most common and easily available from the plantations.

1. *Sympiesis hyblaeae* Surekha (Eulophidae: Hymenoptera) (Fig.1a)
2. *Palexorista solennis* Walker (Tachinidae: Diptera) (Fig.1b).

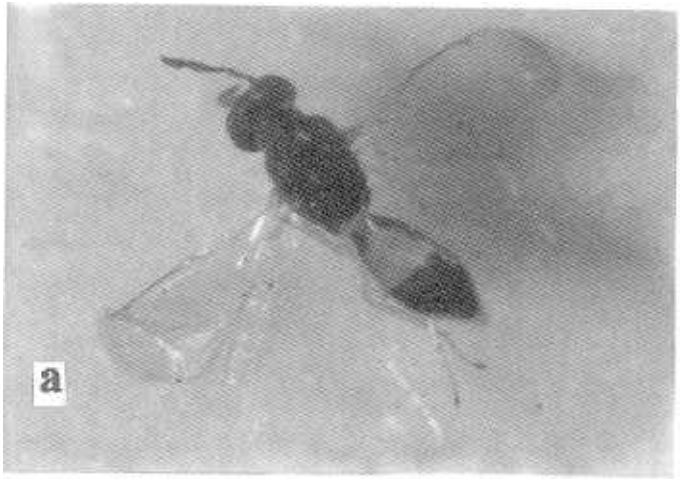
2.2.2. Exotic egg parasitoids

Two species of *Trichogramma,* namely, *T.embryophagum* (Hartig) and *T. dendrolimi* Matsumara were screened against *H. puera.* The nuclear cultures of the parasitoids were obtained from Project Directorate of Biological Control (ICAR), Bangalore and mass multiplied on the rice moth *Corcyra cephalonica* (Staint)as per standard methods (Singh and Jalali. 1994).

2.3. BUILDING UP OF NUCLEAR CULTURE OF INDIGENOUS PARASITOIDS

The cultures of the parasitoids were maintained in the laboratory at a temperature of 28 ± 2°C and RH 75 ± 5 percent.

A nuclear culture of *Sympiesis hyblaeae* was built from immature stages of the parasitoid collected from the teak plantations at Nilambur during September, 1993. The easiest way of collecting immature stage of this parasitoid was from fresh leaf folds on tender teak leaves occupied by I-II instar *H. puera* larva. Irrespective of whether a leaf fold contained parasitised *Hyblaea* or not,



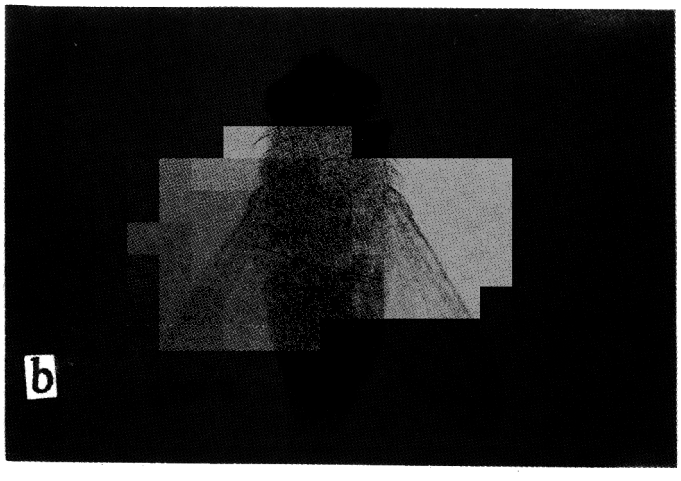


Fig. 1a. Adult *Sympiesis hyblaeae*. b. Adult *Palexorista solennis*.

the leaf folds formed by I-II instar *Hyblaea* larvae were cut using a scissor and transferred to a clean glass tube. The leaf folds were carefully opened in the laboratory and examined for parasitoid larvae. A fresh leaf fold usually contained either a healthy host larva, a parasitised host larva with a parasitoid larva feeding on it or remains of a host larva and a parasitoid larva or pupa. Parasitoid pupae were transferred to a glass tube for emergence of the adults. These adults were used for further multiplication.

A nuclear culture of *P. solennis* was built up from a sample of III-V instar *H. puera* larvae collected in June 1993 from teak plantations at Nilambur. Several batches of ten to twenty Hyblaea larvae were maintained on teak leaves in glass bottles (15 cm x 10 cm) and observed for emergence of parasitoid maggots from larvae dead in due course of development. In addition to this, Pupae of *H. puera* were also collected from field and observed in the laboratory for emergence of parasitoids. In the case of parasitised host stages, maggots of the parasitoid came out from the dead ones and pupated within the rearing cage. The parasitoid pupae were transferred to clean glass bottles (15 cm x 10 cm) for emergence of adults.

2.4. MAINTAINING A CULTURE OF THE HOST INSECT, *HYBLAEA PUERA*

A culture of *H. puera* was maintained in the laboratory at 28± 2^{O}C and RH 75± 5% in order to facilitate mass multiplication of the parasitoids. The following methods were used.

The culture was started from field collected *Hyblaea* pupae. The pupae were brought to the laboratory and maintained in glass bottles (15 cm x 10 cm) covered with cheese cloth. The emerging adults were kept in pairs in separate glass bottles and provided with 10% honey solution. The adults usually mated on the first day of emergence and started laying eggs from the second night onwards. The eggs were laid on the cheese cloth covering the bottles. The cloth containing the eggs was washed in 0.5% sodium hypochlorite solution for 10 minutes. The cloth containing the eggs was kept in glass bottles along with freshly collected tender teak leaves. The larvae on emergence fed on tender teak leaves until they reached the third instar. These larvae were then transferred individually into sterilized glass tubes (2.5 cm x 8 cm) containing an artificial diet (Mathew. *et al.*,1990). The tubes were closed with sterilized cotton plugs. The pupae formed were removed and transferred to sterilized glass bottles (8 cm x 18 cm) for emergence. One of the major handicap in the host rearing was the death of larvae due to bacterial and virus disease. On many occasions the cultures were lost due to infection and mass culturing of the parasitoids was affected by non-availability of sufficient number of appropriate host stages.

3. RESULTS AND DISCUSSION

3.1. SYMPIESIS HYBLAEAE

3.1.1. Adult morphology

Female measures 2.0-2.8 mm in length. Head and body metallic green to blue. Basal gastral tergites with distinct yellow spot medially. Antenna is simple and not branched. Male measures 1.1 1- 1.53 mm. Similar to female in body colour. Antenna branched.

3.1.2. Behavioural observations

3.1.2.1. Mating behaviour

Methods

Pupae of *S. hyblaeae* were collected and kept individually in clean glass tubes (5cm x 2 cm) and observed for emergence of adults.

Sexing of the adults was done based on their size and antennal morphology. Ten sets of freshly emerged females were paired with fresh males and kept under observation. Observations were recorded every half an hour for a period of 6 hours.

Results

Adults involved in mating within 2-4 hours after their emergence. The duration of mating was about 60-90 seconds. Each male mated with several females during their lifespan although females mated only once.

3.1.2.2. Oviposition behaviour

Methods

As a large number of mated adults were not always available at a time to layout a one-time systematic experiment with replicates, observations were made on the oviposition behaviour at different times with different number of parasitoids. The data of several observations were pooled for analysis.

Field observations showed that *S. hyblaeae* prefers to parasitise first or second instar *Hyblaea* larvae. Hence only such larval stages were used in the experiments. To observe the oviposition behaviour a single mated female was kept inside a clean glass tube (5cm x 2 cm) and provided with 2 host larvae in leaf folds. To record fecundity, each female was provided with fifteen, late first instar or early second instar larvae inside a 100 ml conical flask. After 24 h the larvae were transferred to another glass tube and examined whether parasitised or not. The percentage parasitism was recorded. The same female was provided with fresh host larvae every day until its death. The number of eggs laid each day was recorded and the total number of eggs laid during the life span was calculated.

Results

Egg laying started 24-36 hours after mating. Before oviposition, the female entered inside the leaf fold occupied by the host larva. The host larva was immobilised first and a single egg was laid on the lateral side of its body in the intersegmental region. The egg is white, hymenopteriform and measures about 0.02 mm in length.

Fecundity: Of the 50 pairs observed, only 23 pairs lived for more than 10 days and data from these sets were used for analysis. Fecundity during the lifespan varied from 10-19 with an average of 15. The number of eggs laid per day varied from 1-7 with an average of 5. Egg laying occurred continuously for a period of 3 days in the case of 74% of the pairs and 4 days in the case of the remaining pairs. Out of the 23 pairs, 65.2% laid their maximum number of eggs on the first day of oviposition; 17.4% on the second day and 13% on the third day. Only 4.4% laid their maximum number of eggs on the fourth day. Oviposition was not observed beyond this period though the adults were alive for some more days.

3.1.2.3. Developmental biology

The incubation period of the egg is about one day. The larva is ectoparasitic. The neonate larva remains on the host body and feeds on the body juice and grows. Larval period is about 4-5 days. By the time the larva completes feeding, only the skin of the host body will be seen inside leaf fold. Pupation takes place inside leaf fold ensuring full protection. Pupal period is about 5-7 days. Pupa is brown in colour and measures about 1-2 mm in length. The total developmental period from egg to adult is about 10-13 days.

3.1.2.4. Sex Ratio

On an average, the sex ratio of 250 randomly collected adults was found to be 1:2 (Male : Female).

3.1.3. Biological Observations

3.1.3.1. Effect of different food on lifespan of adults

The effect of three types of food, namely, diluted honey, sucrose solution and glucose solution on the longevity of female *S. hyblaeae* was studied. For each treatment 12 females were used. There were three replicates for each treatment. A single individual was kept in a glass tube (5 cm x 2 cm). The food solution was diluted to 10% and provided as minute drops on polythene strips. Fresh food was given twice during the day time. A control set was also maintained in which adults were fed with distilled water. The number of individuals alive was recorded at an interval of 24 h.

Results

The results are presented in Table 1. The average lifespan of adult *S. hyblaea* was the highest (two weeks) when glucose was given as food. Sucrose fed adults lived only for about one week. Adults fed with honey lived for 3-4 days. In the control set adults lived for 1-2 days. As the activities of the females including the oviposition are stretched over a period of more than one week, a food offering a prolonged lifespan is critical in the multiplication of the parasitoid. The data indicates the appropriateness of glucose as an adult food in the laboratory.

Table 1. Effect of different type of food on the longevity of *S. hyblaea*

Type of food	lifespan (days)	
	Range	Mean ± SD*
Glucose	6-15	13.80 ± 2.85
Sucrose	4-8	7.41 ± 1.38
Honey	3-5	3.83 ± 0.38
Control	1-2	1.60 ± 0.04

Mean of three replicates each with 12 adults.

3.1.3.2. Mass rearing trials

Methods

Mass rearing trials were initiated after assessing the fecundity rate per day, number of eggs laid during the lifespan and duration of oviposition. Based on this ten host larvae each were exposed to one pair of parasitoid for

oviposition. Preliminary observations showed that the parasitoid accept only late first instar or early second instar host larvae for oviposition. Hence for mass rearing trials these stages only were used.

For mass rearing of the parasitoid, five mated females and five males were maintained in a glass bottle (15 cm x 10 cm) and fed with 10% glucose solution. Minute drops of food solution were provided on a 2 cm x 10 cm polythene strip and kept inside the rearing bottle. Fifty first or second instar larvae feeding inside leaf folds were released into the bottle. After 24 h the larval folds were transferred to fresh glass containers and observed for parasitism. Immobilised larvae bearing parasitoid egg were transferred to fresh glass containers. Percentage parasitism and percentage adult emergence were recorded. Fresh host larvae were provided in the rearing bottle every day until the death of parasitoids.

Results

The percentage parasitism varied from 48 to 72 with a mean of 56.8 ± 8.04 (Table 2). Parasitoid pupae developed successfully only from 78% of the 168 parasitised host larvae observed. Of the 131 pupae formed, 84% developed into adults. As stated elsewhere, the average number of eggs laid by a female *S. hyblaeae* is 15 of which about 12 (78%) may develop into pupae, of which 10 pupae (84%) may develop into adults. As per the above rough estimate the possible number of progeny produced by a female could be 10 during its lifespan.

Table 2. Parasitism by *S. hyblaeae* under laboratory studies

*Trial No	@Total number of host larvae exposed	% parasitism obtained
1	140	48
2	170	72
3	210	64
4	180	57
5	90	49
6	120	58
7	160	52
8	150	54
	Mean ± SD	56.8 ± 8.04

• Each trial with 5 parasitoid on the first day and with less number on subsequent days depending on the number of parasitoid alive.
*Total number of larvae provided to the parasitoid during its life span @ 10 larvae/parasitoid/day.

3.1.3.3. Search for an alternate host

The non availability of sufficient number of early instar *H. puera* larvae from the laboratory culture always affected rearing of *S.hyblaeae*. As the lifespan of the parasitoid was considerably shorter than that of the host, several parallel culture of the host differing in their age structure would be required to ensure availability of a particular host stage continuously. This was not possible due to various technical reasons. In view of the above difficulties, the feasibility of using *Corcyra cephalonica*, a well known host used in the laboratory rearing of many hymenopteran parasitoids was examined.

A culture of *C. cephalonica* was maintained in the laboratory for rearing *Trichogramma* spp. from which larvae were available for the experiments.

Experiment I: Ten second instar *H. puera* larvae and ten one week old *C. cephalonica* larvae were exposed to five mated *S. hyblaeae* females in a glass bottle (15 cm x 10 cm). While *H. puera* larvae were feeding within leaf folds, *C. cephalonica* larvae were kept inside separate leaf folds. The larvae were recovered after 48 h and examined for parasitism.

Experiment II: Seventeen second instar *Hyblaea* larvae and 3 *Corcyra* larvae were exposed to five *Sympiesis* females.

Results

In the first experiment, all the *Hyblaea* larvae exposed to *S. hyblaeae* were found parasitised. However in the case of *Corcyra*, only 2 of the 7 larvae were parasitised. Neonate larvae fed on *Corcyra* but they did not complete their development. In the second experiment, while 11 out of 17 *H.* puera larvae were parasitised none of the Corcyra larva was parasitised. The results indicate that *C. cephalonica* is not acceptable to *S. hyblaeae* as a host and the parasitism recorded on two of the experimental larvae could be accidental.

3.1.3.4. Effect of continuous rearing on quality of the progeny

Methods

The fecundity and sex ratio of the parasitoids of the parental set was compared with those of first and second filial generation. To start with the fecundity of 5 females of field origin (collected as pupae) was recorded. The

sex ratio of their progeny was also recorded. Similar data pertaining to first and second generations were also gathered.

Results

The results indicate that there is about 50% reduction in the number of eggs laid by the F2 adults in comparison to the parental set (Table 3). With respect to the two parameters observed parental and F1 set behaved similarly. In the case of F2 set, 75% the population was found to be males. The reduction in the fecundity as well as the male domination in the F2 generation could be considered as a major problem in the mass multiplication of the parasitoids. Frequent introduction of field collected females into the laboratory culture could solve this problem.

Table 3. Effect of continuous rearing of *S. hyblaeae* on quality of progeny

Generations	*Total number of eggs laid	Sex ratio of adults (M:F)
Parental set	82	1.0:2.0
F1	78	1.2:2.0
F2	42	3 0:1.0

*eggs laid by 5 females.

3.1.3.5. Diapause

As stated earlier under normal conditions the pupal period ranged from 5-7 days. However, pupae developed during the period, late December to January took a considerably long time to emerge into adults suggesting a diapause in this species. Of the 50 pupae collected on various dates during January from the laboratory culture 94% entered into diapause, and 6% emerged within the normal time. The diapause period ranged from 111 to 156 days with an average of 163 days (Table 4). The diapaused pupae started emerging from the month of May and the peak emergence was noted in June-July (Table 4).

Table 4. Emergence pattern of fifty *S. hyblaeae* pupae collected in the month of January from the laboratory culture

Month	Number emerged*	Diapause period
January	3 (6)	7
February	0	--
March	0	--
April	0	--
May	5 (10)	111
June	15 (30)	123
July	20 (40)	156
Unemerged (dead)	7 (14)	--

(Figure within parentheses is percentage). Diapause and field population of *S. hyblaeae.*

Methods

Fortnightly sampling of the early larval instars of *H.puera* was carried out in teak plantations during 1993-94 to record seasonal trend of parasitism by ***S.*** *hyblaeae.*

Results

The population fluctuation of *S. hyblaeae* in different months during 1993-94 is presented in Figure 2. There was no incidence of parasitism in the months February, April and May. In March *Hyblaea* larvae could not be collected as they were not traceable in the plantations. The presence of parasitism was first noticed in the month of June with an increase in the following months with a significant decline in January.

The absence of parasitism by *S. hyblaeae* during the months February to May can be attributed to diapause. Eventhough about 10% of the diapaused parasitoid pupae would emerged in May the percentage parasitism on the host could be too small to be detected in the samples collected during this period. With the end of diapause period an increase in the parasitoid population is evident in the following months.

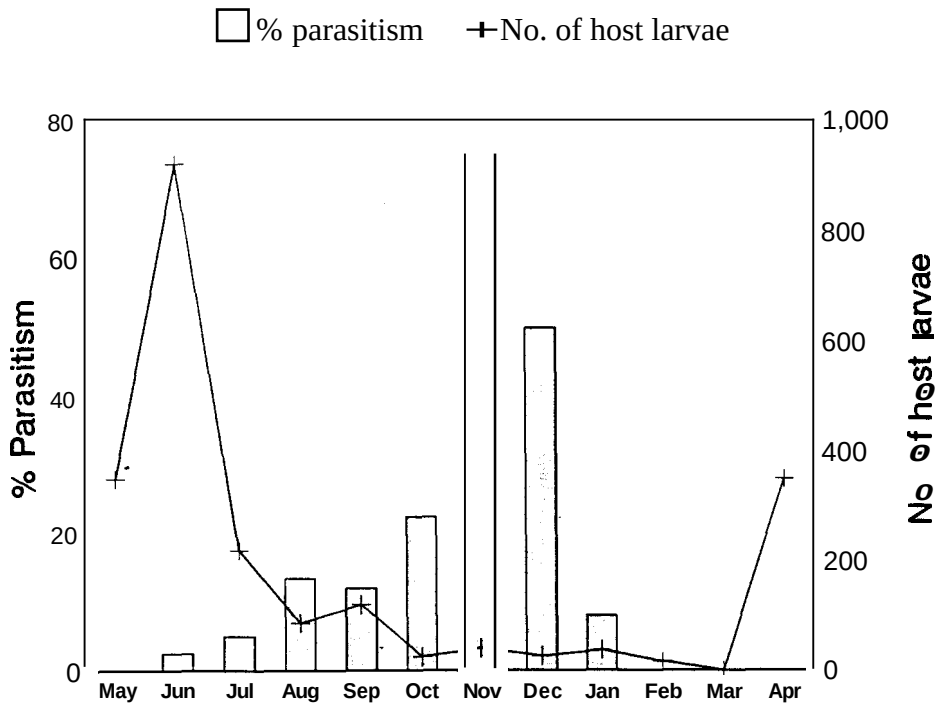


Fig. 2. Parasitism of *Sympiesis hyblaeae on H. puera* during 1993-94. in teak plantations at Nilambur

3.1.3.6. Trials on diapause breaking

Experiments were carried out to establish whether the diapause of *S. hyblaeae* is a genetic phenomenon or it is influenced by the season.

As the peak emergence of the parasitoid is during the rainy months, diapaused pupae were exposed to low temperature and high humidity conditions - a weather condition similar to that of rainy months.

The experiment consisted of three treatments in which five pupae each were exposed to three temperature regimes - 15^{o}C, 25^{o}C and 28^{o}C for a period of three weeks in a BOD incubator. Humidity ranging between 75-85% was maintained within the incubator.

Assuming that the rain during the diapause period could favour emergence of pupae, another experiment was carried out to study the effect of direct wetting of the the pupae on emergence. A set of 25 diapausing pupae (three weeks old) were placed in a petridish and sprinkled with water every day and observed for a period of one week.

Results

None of the pupae used in the first experiment emerged during the period of exposure to low temperature and high humidity. Similarly the wetting of the pupae also did not favour emergence. The result indicates that the diapause in *S. hyblaeae* could rather be influenced by genetic factors than environmental factors.

3.2. *PALEXORISTA SOLENNIS*

3.2.1. Adult morphology

Adult flies emerged in the laboratory culture were sexed according to their morphological characters. The female can be well distinguished by their well developed hypopleural and pteropleural bristles and the prominent post scutellum and the long bristles covering the abdomen. The male has a narrow abdomen with blunt end, covered with minute bristles.

3.2.2. Behavioural observations

3.2.2.1. Mating behaviour

Males emerged earlier than females and freshly emerged females preferred to mate with one day old males. Male exhibited rapid flight movement and finally mounted on the female and grasped her. By slipping backward it extended the tip of the abdomen to that of the female and copulated. Duration of copulation ranged from 20-90 minutes with an average of 31 minutes.

3.2.2.2. Oviposition

The preoviposition period was about two days. Third or fourth instar larvae only were accepted for depositing the eggs. At the time of oviposition the female fly moved swiftly towards the host larva and deposited a single egg on the lateral side of the larva near its prolegs. Very rarely more than one egg was placed on the host body. To oviposit the female stretched its legs and raised the thorax and protruded the tip of the abdomen to the host body. Oviposition did not involve immobilisation of host larva.

Fecundity: Fecundity rate was estimated based on the observation that a female laid a single egg on each host. However very rarely more than one larva developed within a single host. The oviposition period was found to vary from 1-6 days with a peak of 5 days (Table 5 and the number of eggs laid by a female fly during this period varied from 37-55 with an average of 43. Maximum number of eggs were laid during the first two days of oviposition and the remaining eggs in the last four days (Table 6).

Table 5. Frequency distribution of *P. solensis* according to the duration of oviposition (Days)

No. of females	Duration of oviposition
4	1
17	3
28	4
53	5
5	8

Table 6. Progress of fecundity during the oviposition period

Day of oviposition	Average number eggs laid*
1	13
2	12
3	9
4	5
5	3

*Average of 20 females.

The maximum number of eggs laid on a day (i.e. number of hosts parasitised) varied from 5-13 with an average of 9 (Mean of 20 adults).

3.2.3. Developmental biology

The eggs are white in colour and elliptical in shape. Incubation period ranges from 6 to 8 hours (Mean 4.32 ± 1.15 h). The maggot is endoparasitic. The neonate maggot enters into the host body and adheres to the crop wall and sucks the crop fluid. After feeding for about 4-6 days (Mean 4.8 ± 0.45 days) the maggot breaks open the host body and comes out for pupation. Pupation is completed within a day. The pupa is coarctate, brown in colour and measures 4-5mm in length. The pupal period lasts for about 5 to 7 (Mean 5.9 ± 0.5) days. The sex ratio of 75 adults emerged from the laboratory culture was 1:2 (M:F).

3.2.4. Biological observations

3.2.4.1. Lifespan of adults

Effect of different food on lifespan of adults

The effect of three types of food, namely, diluted honey, sucrose solution and glucose solution on the longevity of female *S. hyblaeae* was studied. For each treatment 12 females were used. There were three replicates for each treatment. A single individual was kept in a glass tube (5 cm x 2 cm). The food solution was diluted to 10% and provided as minute drops on polythene strips. Fresh food was given twice during the day time. A control set was also

maintained in which adults were fed with distilled water. The number of live individuals was recorded at an interval of 24 h.

Results

The results are presented in Table 7. The average lifespan of adult *P. solennis* was 18.6 days when fed with honey. Sucrose fed adults lived for 12 days on an average. The lifespan was least when fed with sucrose (5 days on an average). In the control set fed with distilled water adults lived for 1-2 days. The data indicates the appropriateness of honey as the adult food in the laboratory.

Table 7. Effect of different type of food on the longevity of *P. solennis*

Type of food	lifespan (days)		
	Range	Mean*	± SD
Honey	5-24	18.63	± 7.7
Glucose	2-13	12.08	± 3.2
Sucrose	2-9	5.00	± 2.5
Control	1-2	1.60	± 0.04

* Mean of three replicates each with 12 adults.

3.2.4.2. Mass multiplication trials

Methods

Based on the past field observations (Sudheendrakumar, 1986),late third or early fourth instar larvae were used for rearing *P. solennis* in the laboratory. The rearing was carried out at 27± 2^{O}C and RH 65 ± 5%.

Mated females and males were grouped in the ratio 3:2 and released in a wooden cage with glass sides (30 cm x 30 cm x 30 cm). Honey (10%)solution was provided on cotton swabs as food. Thirty *H. puera* larvae feeding on teak leaf were exposed to the flies in the cage. After 24h of exposure to the parasitoids, the larvae were transferred to individual glass tubes (15 cm x 10 cm) and observed for parasitism. Larvae which were found alive at the time of collection were only used for further observation on emergence of parasitoid. The same set of flies were again provided with fresh host larvae on the second day at the rate of 10 larvae/parasitoid until their death. The

total number of larvae provided in a cage per day varied depending on the number of live parasitoid female present in the cage.

Data on percentage parasitism, percentage pupation and adult output were recorded.

Results

Continuous rearing of *P. solennis* on *H.puera* was feasible. The success rate of rearing is presented in Table 8. Data from six different trials showed that the percentage of parasitism ranged from 33.3 to 75 with a mean of 55 ae 16.1. Parasitoid pupae developed from 81.8% of the parasitised host larvae. However, adults emerged from only 41.5 ae 10.08% of the parasitised larvae.

Table 8. Parasitism, pupal output and adult emergence of *P. solennis* from parasitised *Hyblaea* larvae

Trial No.	No. of parasitised larvae observed	% parasitism	% pupation	% adult formed
1	90	62.2	78.6	31.8
2	72	44.4	96.9	48.4
3	78	69.2	100.0	55.6
4	84	33.3	85.7	28.6
5	96	75.0	58.3	41.7
6	120	46.7	71.4	42.9
	Mean ± SD	55.1 ± 16.1	81.8 ± 15.8	41.5 ±10.1

Each trial with 3 females.

3.2.4.3. Development of an artificial diet

Composition of *P. solennis* artificial diet

Casual observations showed that *P. solennis* larvae can feed and survive in a diet consisting of of agar and glucose in distilled water. However, the developmental period of the larvae in this medium was found to be considerably longer - about three weeks. Also the larvae never entered into pupation. The diet was then improved by adding fresh pupal extract and a composition was developed after a series of trials (Table 9).

Table 9. Composition of the artificial diet for rearing *P. solennis*

Item No.	ingredient*	Quantity
1	Agar	10 gm
2	Yeast extract	5 gm
3	Casein purified	20 gm
4	Glucose	10 gm
5	Sucrose	4 gm
6	Streptomycin sulphate	250 mg
7	Multivitamine mineral mixture	1 caps
8	Vitamin E	2 gm
9	*H. puera* pupal extract	10 pupae
10	Formaldehyde 10% solution	1 ml
11	Distilled water	700 ml

* ingredients for 1 litre diet.

The ingredients were added to distilled water and thoroughly blended in a domestic mixer and stored in refrigerator.

Inoculation of larvae in the medium

Though for the initial trials partially grown-up larvae emerged from the host larvae were used, methods were later standardised to rare the parasitoid larvae right from their emergence from the eggs.

Egg collection

Mated females which completed their preoviposition period were selected for collection of eggs. Each female was dissected in a petri-dish containing distilled water and the eggs from the ovary were released. The eggs before hatching were transferred to a clean petridish containing the liquid diet. The larvae were shifted to fresh diet every day. Initially 10-20 larvae were reared together and after the third day only about 5 larvae were reared per petri-dish (Fig. 3).

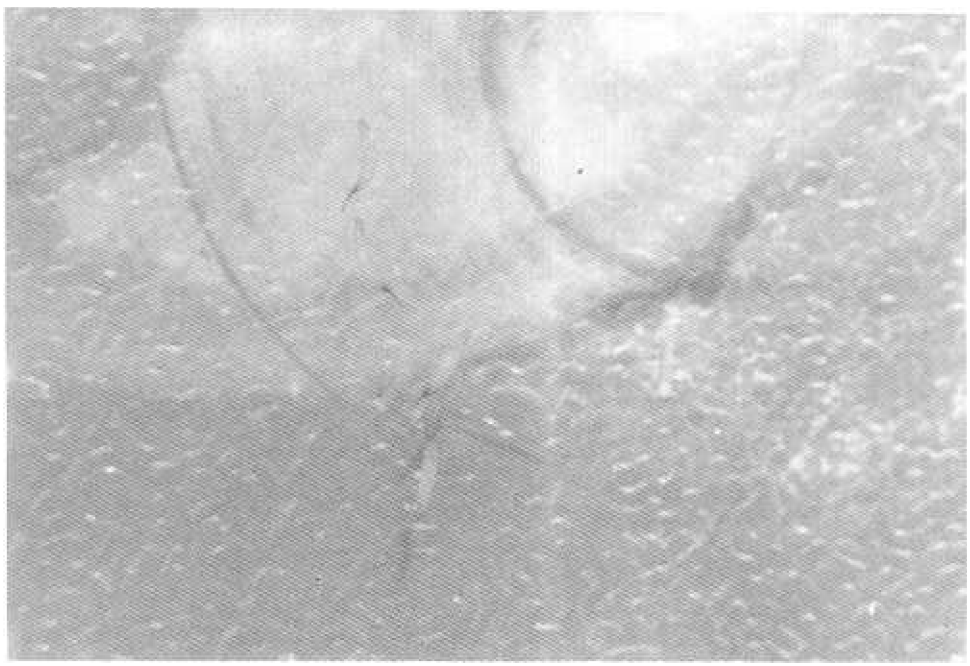


Fig. 3. *P. solennis* larvae growing in the artificial diet

The number of eggs collected from each female was recorded. The number of eggs hatched, pupae formed and adult emerged were recorded. Data on total developmental period was also collected.

Results

The ovarial dissection of 25 females showed that egg yield from a gravid female varied from 16-33 with an average of 24.4 ± 6.32 (Table 10). While 38.2% (Range22.5 - 68.1%) of the larvae fed with artificial diet developed into pupae, 59.8% (Range 28.6 - 100%) of the pupae developed into adults (Table 11). However, on the whole only 25.9 ± 11.8% (Range 6.5-45.5) the larvae fed with artificial diet developed into adults (Table 12 The adults formed were normal and comparable with the adults reared on natural host.

Table 10. Yield of eggs from *P. solennis* females on ovarial dissection

Batch No.	No. of females dissected	No. Of eggs collected	Average egg yield
1	5	80	16
2	1	22	22
3	1	31	31
4	2	52	26
5	1	32	32
6	4	72	18
7	1	33	33
8	5	100	20
9	5	120	22
	Mean ± SD		24.4 ± 6.32

Table 11. Pupation and adult formation of the larvae reared in the diet

Trial No.	No. of larvae reared in the diet	% pupated	% pupae developed to adult	sex ratio (M:F)
1	61	24.6	100.0	1.0:2.0
2	61	49.1	56.7	1.1:1.0
3	62	30.6	52.6	2.3:1.0
4	62	58.0	58.3	1.0:2.0
5	22	68.1	46.7	1.0:2.5
6	31	22.5	28.6	0:2.0
7	52	23.0	58.3	1.0:2.0
8	32	65.6	57.1	1.0:1.4
9	72	34.7	68.0	1.1.1.0
10	33	63.6	71.4	1.0:1.5

Table 12. *P. solennis* adult output from an artificial diet based culture

Trial No.	No. of larvae reared in the diet	% of adults formed
1	61	24.6
2	61	26.1
3	62	16.0
4	62	33.4
5	22	31.8
6	31	6.5
7	52	13.5
8	32	37.5
9	72	23.6
10	33	45.5
Mean ± SD	48.8 ± 17.5	25.85 ± 11.76

The oviposition period was comparable with that of host reared adults which varied from from 1-6 days and the number of eggs laid by a female during this period varied from 15-33 with an mean of 24.8± 5.6. (Table 13). The sex ratio of the adults formed in the artificia1 diet was found to be 1: 1.6 (M:F) on an average.

Table 13. Fecundity of *P. solennis* reared on artificial diet

Sl. No. of female	Number eggs laid
1	33
2	30
3	31
4	26
5	25
6	25
7	23
8	19
9	15
10	21
Mean ± SD	24.8 ± 5.6

A comparative account of the efficiency of rearing *P. solennis* on artificial diet and host larva is presented in Table 14. In the case of larvae developed on host insect, the percentage pupation was 81.8 as against 38.2% in the artificial diet. Similarly adult formation was on the higher side (41.5%)

when the insect was reared on the natural host. In the case of parasitoid larvae developed on host insect, the larval period was shorter than those developed on artificial diet. However, the pupal period and the sex ratio did not vary between the two methods of rearing. The mean fecundity of diet reared females was 24.8 as against 43 in the case of host-reared females.

Table 14. Comparative efficacy of rearing *P. solennis* on host larva and artificial diet

Parameters	Rearing method	
	on host larva	on diet
Per cent larvae pupating	81.8	38.2
Per cent larvae developing into adults	41.5	25.9
Larval period (days) (Mean ± SD)	4.8 ± 0.45	6.0 ± 0.72
Pupal period (days) (Mean ± SD)	5.9 ± 0.5	6.0 ± 0.75
Sex-ratio (M:F)	1:2	1:1.6
Average fecundity	43	25

Attempts have been made in the past to develop artificial diet for rearing tachinid parasitoids. Bratti and Nettles (1989) developed a diet containing haemolymph of the sphingid moth, *Manduca sexata* for rearing *Palexorista laxa* a parasitoid of *Helicoverpa armigera* the result of which was encouraging. Their diet also contained soybean residue which served as a nutrient for the larva. Mellini et *al.*, (1994) reported that *Exorista larvarum* L. (Diptera) reared on an artificial diet based on bovine serum (75%) and extract of *Galleria mellonella* (Lepidoptera) pupae (20%) and other additives (5%) produced individuals comparable to those reared on its natural host, in their quality and developmental period.

The agar based artificial diet developed for rearing *P. solennis* appears to be successful though it is not superior to the natural host. Hence further improvement of the diet is required to enhance the success of rearing.

3.3. EXOTIC PARASITOIDS

3.3.1. Screening of exotic egg parasitoids

Methods

In the present study, acceptance of *H. puera* eggs by two exotic species of *Trichogramma,* viz.,*T. embryophagum* (Hartig) and *T.dendrolimi* Matsumara were studied. These two species were selected considering their arboreal nature and their ability to reach considerable heights on the top of tree crops like teak.

A culture of Hyblaea puera was maintained in the laboratory (See Section 2). In the the oviposition cage (Glass bottle of 15 cm x 10 cm) female moths deposited eggs on the innerside of the cotton cloth used for closing the mouth of the bottle. The cloth holding the eggs glued on it was soaked in distilled water and using a camel brush the eggs were separated. White paper cards of **3** cm x 1 cm were cut and cards holding eggs @ 4,l0 and 20 were prepared by gluing eggs to the card.

Two experiments were carried out. (1)Effect of different parasitoid - host egg ratio on percent parasitism. (2) Effect of age of host egg on percentage parasitism.

Under the first experiment, the parasitoid - host egg ratio tested were 1:4, 1:10 and 1:20. Each card was exposed to a single mated female in a glass vial (10 cm x 3 cm). There were 10 replicates for each treatment. After 24 h the card bearing the eggs were taken out and the eggs were observed. By next day parasitised eggs developed a black colour due to development of parasitoid larva inside. Percentage parasitism in each treatment was recorded.

In the second experiment on the effect of age of eggs, fresh eggs and one day old eggs were exposed at the rate of 10eggs to one mated female for 24 hours. For each treatment ten replicates were kept. Percentage of parasitism under the two treatments were recorded.

The data were transformed into corresponding angles and (arc age) and subjected to analysis of variance using SPSS statistical package.

Results

The result of the first experiment indicated that percentage parasitism was significantly different between the two parasitoid species and between the three parasitoid-host ratio. However, the interaction between the parasitoid

and the parasitoid-host ratio was non significant. Parasitism caused by *T. embryophagum* was significantly higher than that of *T. dendrolimi* and highest parasitism occurred under the parasitoid-host ratio of 1:4 (Table 15).

Table 15. Parsitisation of *T.dendrolimi* and *T. embryophagum* under different parasitoid-host egg ratio

Parasitoid	Mean percentage parasitism			
	Parasitoid-host egg ratio			
	1:4	1:10	1:20	Mean
T. dendrolimi	95.0 (75.23)	80.0 (55.08)	60.5 (38.2)	78.5 (56.17)
T. embryophagum	82.5 (60.05)	63.0 (39.32)	58.5 (36.11)	68.0 (45.16)
Mean	88.8 (67.64)	71.5 (41.19)	59.5 (37.16)	

Figure in parentheses indicates angles corresponding to percentage.

C.D. : = 0.05)

Parasitoid : (16.36)
Parasitoid-host ratio : (12.74)
Interaction : not significant

Table 16. Effect of age of host egg on percentage parasitism

Parasitoid	Percentage parasitism	
	Age of egg	
	Fresh	One day old
T. dendrolimi	75.0 (54.32)	13.0 (7.49)
T. embryophagum	60.0 (37.01)	17.0 (9.81)
Mean	67.5 (45.66)	15.0 (8.85)

Figure in parentheses indicates angles corresponding to percentage.

C.D. : (P = 0.05)

Parasitoid : (19.16)
Parasitoid-host ratio : (33.46)
Interaction : (33.46)

The second experiment indicated that the percentage parasitism was significantly higher on fresh eggs than on one day old eggs. Percentage parasitism caused by *T. embryophagum* was significantly higher than that of *T. den-*

The interaction between the age of the egg and the parasitoid species was also significant (Table 16).

4. GENERAL DISCUSSION

4.1.1. Indigenous Parasitoids

An attempt is made here to evaluate the usefulness of the parasitoids based on their biological characteristics generated in this study.

4.1.1.1. *Sympiesis hyblaeae*

The members of the genus *Sympiesis* Forster are in general parasitoids of lepidopteran leaf miner. The two other *Sympiesis* spp. reported from India include *S. dolichogaster* Ashmead on ground nut leaf miner, *Aproaerema modicella* (Lepidoptera: Gelachiidae) (Shekharappa. et *al,* 1990). the tea leaf miner, *Caloptilia theivora* Walsingham (Lepidoptera: Gracillaridae) (Selvasundaram and Muraleedharan 1987) and an unidentified species on cashew leaf miner, Acrocercops syngramma Meyr. (Sundararaju, 1984).

Attempts have been made in the past to use *Sympiesis* species in biological control. A classical example is the success achieved in the control of *Promecotheca cumingi* Baly (Coleoptera: Hispidae), the coconut leaf miner in Sri lanka using *S. javanicus* (Ferriere) imported from Singapore and Borneo (Dharmadhikari *et al.,* 1977) *Sympiesis* species in general act as co-parasitoids along with the other similar parasitoid species and contribute to the reduction of host insect which is true also in the case of *S. dolichogaster* (Shekharappa. *et al,* 1990: Selvasundaram and Muraleedharan, 1987).

S. hyblaeae is monophagous and as a parasitoid infesting the very early larval stage, this species is of great importance from the point of its ability to kill the pest before it causes economic damage. However, In teak plantations the parasitoid was found not active during the months of pest outbreaks. The study revealed that a very high proportion of the *S. hyblaeae* population remained in diapause during the early period of pest build-up in April-May and the parasitoid could not numerically respond to the increasing pest population. Their inactivity during the pest build-up period could thus be explained.

The feasibility of mass multiplication is an important criteria in selecting a parasitoid for biological control programme. Mass multiplication of *S. hyblaeae* was affected by its diapause and the feasibility of producing large

numbers of the parasitoid for releasing in the field during the critical period of pest outbreak appeared to be limited. Though preliminary trials carried out to break the diapause were not successful further studies in this line could be useful.

4.1.1.2. *Palexorista solennis*

Tachinid flies with their high fecundity and ability to disperse offer good scope for biological control. Among the indigenous species reported to be partially successful includes *Sturmiopsis inferens* Tns. against the sugarcane shoot borer Chilo infuscatellus Snellen (David and Easwaramoorthy,1987).*Palexorista solennis (= Sturmia inconspicuella* Bar.) is a polyphagous species and is known to parasitise about 23 species of lepidopterans (Chatterjee and Misra, 1974).

Earlier studies have shown that the population of *P. solennis* in comparison with the outbreak population of *H. puera* during the period of April-June is very small and therefore not effective in bringing down the pest population (Sudheendrakumar, 1986).Mass release of such a parasitoid during the pest outbreak period could be beneficial to control the pest.

The present study revealed that mass multiplication of *P. solennis* on *H. puera* in the laboratory is feasible. However, during the study period difficulty in maintaining a continuous culture of the host insect due to disease incidence affected mass multiplication of the parasitoid. The feasibility of rearing this insect on an artificial diet as established in this study is an answer to the mass multiplication of the parasitoid without depending on the natural host insect. However, further improvement of the diet is required for better results.

4.1.2. Exotic egg parasitoids

The trichogrammatid egg parasitoids are considered to be one of the most effectivebiocontrol agents as they infest host eggs and destroy the pest at the very beginning of its infestation. In India about 26 trichogrmmatids are known to be parasitoids of many lepidopterous pests of agricultural, horticultural and forestry crops (Singh and Jalali, 1994).

Ahmad (1989) reported acceptance of *H. puera* eggs by three species of Trichogramma namely *T. chilonis* (= australicum) Ishii, *T. brasiliensis* Ashmead and *T. japonicum* Ashmead. The present study showed that *H. puera* eggs are susceptible to parasitism by *T. dendrolimi* and *T. embryophagum* under laboratory conditions. In terms of percentage parasitism the latter

species is superior to the former. Both the species preferred to parasitise fresh eggs and percentage parasitism on one-day old eggs was significantly lower than that on fresh eggs. This suggested that successful parasitism by these parasitoids was depended on the availability of the parasitoids in the field when the eggs were fresh. It is concluded that a delay in releasing the parasitoid could result in a low level parasitism as the incubation period of *H. puera* eggs is less than two days.

T.embryophagum and *T.dendrolimi* appears to be promising biological control agents. However their efficacy under field condition needs to be evaluated. Further success of using them against *H. puera* depends on our ability to predict the pest incidence and their timely release in the field.

5. CONCLUSIONS

The feasibility of mass rearing is an important criteria for selecting an indigenous parasitoid as a biological control agent.

Of the two parasitoids studied, *Palexorista solennis* merits attention as a biocontrol agent for practical use as this species can be continuously multiplied on natural host as well as on an artificial diet and used for inundative release.

The mass multiplication of *Sympiesis hyblaeae* was affected by its diapause during the months, February to May. This suggests that the parasitoid cannot not be made available in large numbers during the months April-May which is the critical period of pest outbreak. It is thus concluded that the scope of using this species as a biocontrol agent is limited.

Both species of the exotic egg parasitoids, *Trichogramma embryophagum* and *T. dendrolimi* accepted *H. puera* eggs. However, their preference for fresh eggs suggests that for successful parasitism, the time gap between their release in the field and the egg laying by *H. puera* should not exceed 24 hours. As a prerequisite for using the egg parasitoids. an accurate pest outbreak prediction system needs to be developed for timing of the release of the egg parasitoids.

7. REFERENCES

Ahmad, M. 1989. Potential of egg parasites, Trichogramma spp., against defoliator pest of teak, *Tectona grandis. In*: Proceedings of the Seminar on plant protection, 1989, FRI. Dehra Dun: 1-4.

Beeson, C.F.C. 1941. The Ecology and Control of the Forest Insects of India and the neighbouring countries. Govt. of India (1 96 1 reprint) : 761p.

Bratti, A. and Nettles, W.C. 1989. Invitro rearing of *Palexorista laxa* (Curran)(Diptera: Tachinidae) on haemolymph based diet. Universita - degli- Studi-di-Bolgona, 43: 23-30.

Chatterjee, P.N. and Misra, M.P. 1974. Natural enemy and plant host complex of forest insect pests of Indian region. *Indian For. Bull*., 265: 33p.

David, H. and Easwaramoorthy, S. 1987. Recent advances in biological control of sugarcane pests. In: K.J. Joseph and U.C. Abdurahiman (Eds.). Advances in biological control research in India. Department of Zoology, University of Calicut: 200-213.

Mathew, G.; Sudheendrakumar, V.V.; Mohanadas. K. and Nair, K.S.S. 1990. An artificial diet for the teak defoliator, *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae). *Entomon.* 15(3 & 4): 159-163.

Mellini, E.: Campadelli. G. and Dindo, M.L. 1994. Artificial culture of Exorista larvarum L (Diptera: Tachinidae). Bullettino-dell' Istiuto-di-Entomologia-Guido-Grandi-della - Universita - degli- Studi-di-Bolgona, 48: 1- 10.

Nair, K.S.S.; Mohanadas. K. and Sudheendrakumar. V.V. 1994. Biological control of the teak defoliator, *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae) using insect parasitoids - Problems and prospects. In: T.N. Ananthakrishnan (Ed.). Biological control of social forest and plantation crops insects. Oxford & IBH. 75-95.

Nair, K.S.S.: Sudheendrakumar. V.V.; Vanna. R.V.; Chacko. K.C. and Jayaraman, K. 1996. Effect of defoliation by *Hyblaea puera* and *Eutectona machaeralis* (Lepidoptera) on volume increment of teak. In: K.S.S. Nair. J.K. Sharma and R.V. Varma (Ed.). Proceedings of the IUFRO Symposium, KFRI, Peechi (1993): 257-273.

Nair, K.S.S.: Sudheendrakumar. V.V.; Vanna. R.V.; Chacko. K.C. 1985. Studies on the seasonal incidence of defoliators and the effect of defoliation on volume increment of teak. KFRI Research Report No. 30, KFRI, Peechi, Kerala. 78p.

Selvasundaram, R. and Muraleedharan, N. 1987. Life history and seasonal abundance of *Sympiesis dolichogaster* Ashmead (Hymenoptera: Eulophidae) a larval parasitoid of the tea leaf roller. In: K.J. Joseph, and U.C. Abdurahiman (Eds.). Advances in biological control research in India. Department of Zoology, University of Calicut: 106-109.

Shanower. T.G.; Wightman, J.A.; Gutierrez, A.P. and Rao. G.V.R. 1992. Larval parasitoids and pathogens of groundnut leaf miner, *Aproaerema modicella* (Lepidoptera: Gelachiidae) in India. *Entomophaga,* 37(3): 419-427.

Shekharappa; Somasekhar and Patil, B.V. 1990. Parasitoid complex of groundnut leaf miner, *Aproaerema modicella* Deventer (Lepidoptera:Gelachiidae). *Journal of biological control,* 4(1): 55-56.

Singh, S.P. and Jalali. S.K. 1994. Trichogrammatids, Technical Bulletin, No.7, Project Directorate of Biological control (ICAR), Bangalore, 93p.

Sudheendrakumar, V.V. 1986. Studies on the natural enemies of the teak pests, *Hyblaea puera* and *Eutectona machaeralis.* KFRI Research Report No.38, 23 p.

Sudheendrakumar. V.V. 1990 A report on the ichneumonid parasite of *Hyblaea puera* recorded from Nilambur. Kerala. *J. Trop. Forestry,* 6(1): 102- 103.

Sundararaju, D. 1984. Cashew pest and their natural enemies in Goa. *Journal of plantation Crops,* 12(1): 38-46.

K. Surekha; J. Lasalle; V.V. Sudheendrakumar and S.T. Murphy 1996. A new species of *Sympiesis* (Hymenoptera: Eulophidae) parasitic on the teak defoliator, *Hyblaea puera* (Lepidoptera: Hyblaeidae) in India. *Bull Ent. Res.*, 86: 73-76.