Seasonal variation in infestation characteristics of bagworm (Lepidoptera: Psychidae) in avenue plantation of *Acacia* and *Peltophorum* in the Chhattishgarh region

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Seasonal infestation profile of bagworm, *Eumeta crameri* was constructed based on data recorded over a time scale of twelve consecutive months in the Chhattishgarh region. Two plantation sites consisting of 433 *Acacia nilotica* and 517 *Peltophorum pterocarpum* trees were chosen for the study. Plants were examined at monthly intervals and a visual score assignment system was employed to determine the degree of infestation. Cosinor technique was used to validate statistically significant annual rhythm in infestation characteristics. Results reveal that in both sites maximum outbreak of infestation occurred between November and January in the form of a single peak in each infestation parameter, notwithstanding evidence in favour of two additional generations during the study.

BAGWORMS are a group of highly specialized Lepidopterans belonging to the family Psychidae[1]. There are about 40 species available in the Indian territory[2]. Extreme development of sexual dimorphism in bagworm makes it a biological curiosity. The male bagworm moth is winged whereas the female lacks functional appendages. It spends the most part of its life inside a cylindrical/fusiform bag, which is open at both ends. The bag is constructed by the larva by using silken thread and other extraneous materials, such as leaves, spines, stems and twigs. Until pupation the larva keeps itself on the move from one branch to another in the host plant along with the bag, which is usually referred to as a mobile nest or case.

The bagworm has been documented as a serious defoliator of ornamental shrubs and trees throughout the Southeast and lower Midwest[3]. The potentiality of bagworm as a pest has been studied by several workers[4-7]. In India, until recently bagworms were not recognized as serious pests[2,4]. It has now been clearly established that many species of bagworms have already attained the status of a serious pest in Kerala.

Pteroma plagiophleps (Hampson) causes large scale defoliation of forest plantations of *Albizia falcateria* and *Delonix regia*[8,9]. However, very little is known about the bagworm species prevalent in this country. In this communication we document the prevalence of a population of bagworm species, *Eumeta crameri* and its infestation characteristics in selected avenue plantation sites in the Chhattishgarh region. In addition, seasonal infestation profile of *E. crameri* has been examined.

The *Acacia nilotica* plantations along the NH #6 passing through Bhilai (lat. 20°10′N; long. 81°20′E) and the avenue plantation of *Peltophorum pterocarpum* in the campus of Bhilai Steel Plant were chosen for the study. Plants in both sites with $n = 433$ and $n = 517$ respectively, were examined for bagworm infestation every month for a period of 12 months. A visual score assignment system was employed to determine the degree of infestation[10]. A plant was assigned a score of S_0, S_1, S_2, S_3 or S_4 representing respectively, no, low, medium, high and very high level of infestation. While the low level of infestation was characterized by the presence of at least one bag visible from the ground, the very high level was marked by sizable amount of defoliation[7]. The monthly intensity of infestation was computed and expressed as the ratio of number of plants in each score to the total number of plants, for example $I_1 = S_1/n$ (see Table 1).

Analysis of variance was used to validate statistically significant seasonal effect. In addition, an annual rhyth-

Table 1. Monthly infestation profile of bagworm in the avenue plantations of *Acacia nilotica* and *Peltophorum pterocarpum*

Month	Infestation intensity				
	I_1	I_2	I_3	I_4	I_t
Acacia nilotica					
September	0.127	0.362	0.434	0.016	0.939
October	0.099	0.385	0.457	0.009	0.95
November	0.131	0.401	0.434	0.004	0.97
December	0.097	0.420	0.436	0.006	0.959
January	0.078	0.411	0.454	0.006	1.651
February	0.212	0.413	0.281	0.027	0.933
March	0.295	0.376	0.226	0.050	0.947
April	0.314	0.258	0.217	0.080	0.869
May	0.143	0.210	0.154	0.205	0.712
June	0.110	0.189	0.124	0.374	0.797
July	0.212	0.235	0.256	0.041	0.744
August	0.272	0.212	0.184	0.000	0.668
Peltophorum pterocarpum					
September	0.265	0.228	0.166	0.003	0.612
October	0.245	0.216	0.156	0.003	0.62
November	0.235	0.208	0.116	0.003	0.562
December	0.218	0.162	0.148	0.002	0.53
January	0.294	0.236	0.199	0.003	0.732
February	0.247	0.286	0.156	0.007	0.696
March	0.274	0.267	0.19	0.013	0.744
April	0.197	0.224	0.185	0.029	0.635
May	0.158	0.189	0.168	0.073	0.588
June	0.114	0.156	0.133	0.241	0.644
July	0.208	0.170	0.147	0.013	0.538
August	0.181	0.156	0.119	0.002	0.458

$I_1 = S_1/n$ (ratio of the frequency of plants which were assigned S_1 and total number of plants in the site); $I_2 = S_2/n$; $I_3 = S_3/n$; $I_4 = S_4/n$. $I_t = I_1 + I_2 + I_3 + I_4$.

mic variation for all the levels of infestation was characterized for both sites by the parameters of the best fitting cosine function approximating all data[11]. The rhythm parameters estimated by this least squares method include the mesor (M, rhythm adjusted annual mean), the amplitude (A, half of the difference between minimum and maximum of the best fitting cosine function) and the acrophase (ϕ, time of the annual peak obtained from this cosine function with local midnight of December 31 as ϕ reference). A rhythm is detected, with regard to the considered τ ($\tau \equiv 365.25$ days) when the amplitude differs from zero (non-null amplitude test) with $P \leq 0.05$. This single cosinor method[12] was employed for the original time series and also for the series obtained after fitting 3-item moving average. The latter was employed in order to smoothen the annual curve in the intensity of infestation[13]. Other conventional statistical techniques were also followed whenever required[14].

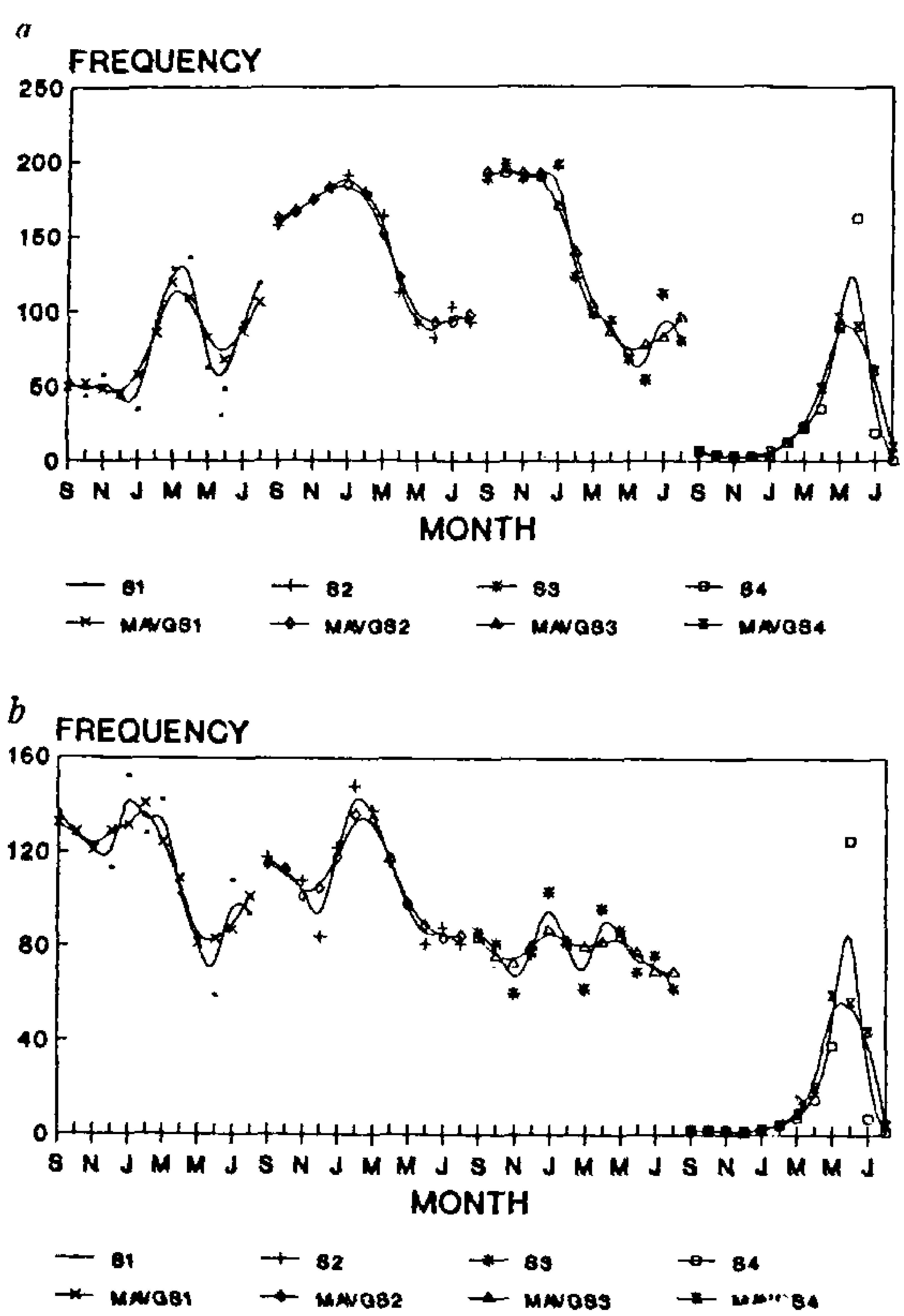


Figure 1. Seasonal variation in the levels of bagworm infestation in plantation sites of (*a*) *Acacia nilotica* and (*b*) *Peltophorum pterocarpum*. S_1, S_2, S_3 and S_4 denote original data indicating low, medium, high and very high level of infestation, respectively. $MAVGS_1$, $MAVGS_2$, $MAVGS_3$ and $MAVGS_4$ denote 3-item moving average fitted data of the time series, S_1, S_2, S_3 and S_4 respectively.

The seasonal variation in intensity of infestation was examined over a period of twelve months. Both original and 3-item moving average fitted time series were plotted as a function of the time of the year (Figures 1 *a,b*). The monthly intensity of infestation was gauged by computing the ratio of number of plants in each score to the total number of plants. The sum of all the ratios in each month was computed and expressed as the total infestation intensity (Table 1). A statistically significant month effect was validated by ANOVA only for I_4 level of infestation intensity (Table 2). The masking of month effect for other intensity of infestation levels could be because of the disparity in infestations between *P. pterocarpum* and *A. nilotica* leading to neutralization of such effect. However, a highly statistically significant species effect was documented for all the levels of infestation intensity excluding I_1 (Table 2). The results reveal that *A. nilotica* was more heavily infested with bagworms than *P. pterocarpum*. Table 3 shows the results of cosinor analysis employed on 3-item moving average fitted time series. A statistically significant annual rhythm was validated for almost all the infestation parameter, excepting S_3 in *P. pterocarpum*. The annual peaks for $S_1 + S_2 + S_3$ infestation intensity occurred on November 27 and December 27 in *Acacia* and *Peltophorum* plantations respectively (Table 3). The peak map is shown in Figure 2, which indicates that 95% confidence limits in peaks for most of the infestation parameters, irrespective of host plants, overlap with each other.

This is the first study that concerns bagworm infestation in the Chhattishgarh region. As such there are limited centers in India where work of comparable nature is being carried out. Prominent among these is the Kerala Forest Research Institute at Peechi. Until 1981 the bagworm, *Pteroma plagiophleps* was known as a minor pest of *Tamarindus indica* in India, when Nair *et al.*[8] demonstrated that *P. plagiophleps* has already assumed

Table 2. Summary of the results of ANOVA* employed to analyse seasonal infestation intensity

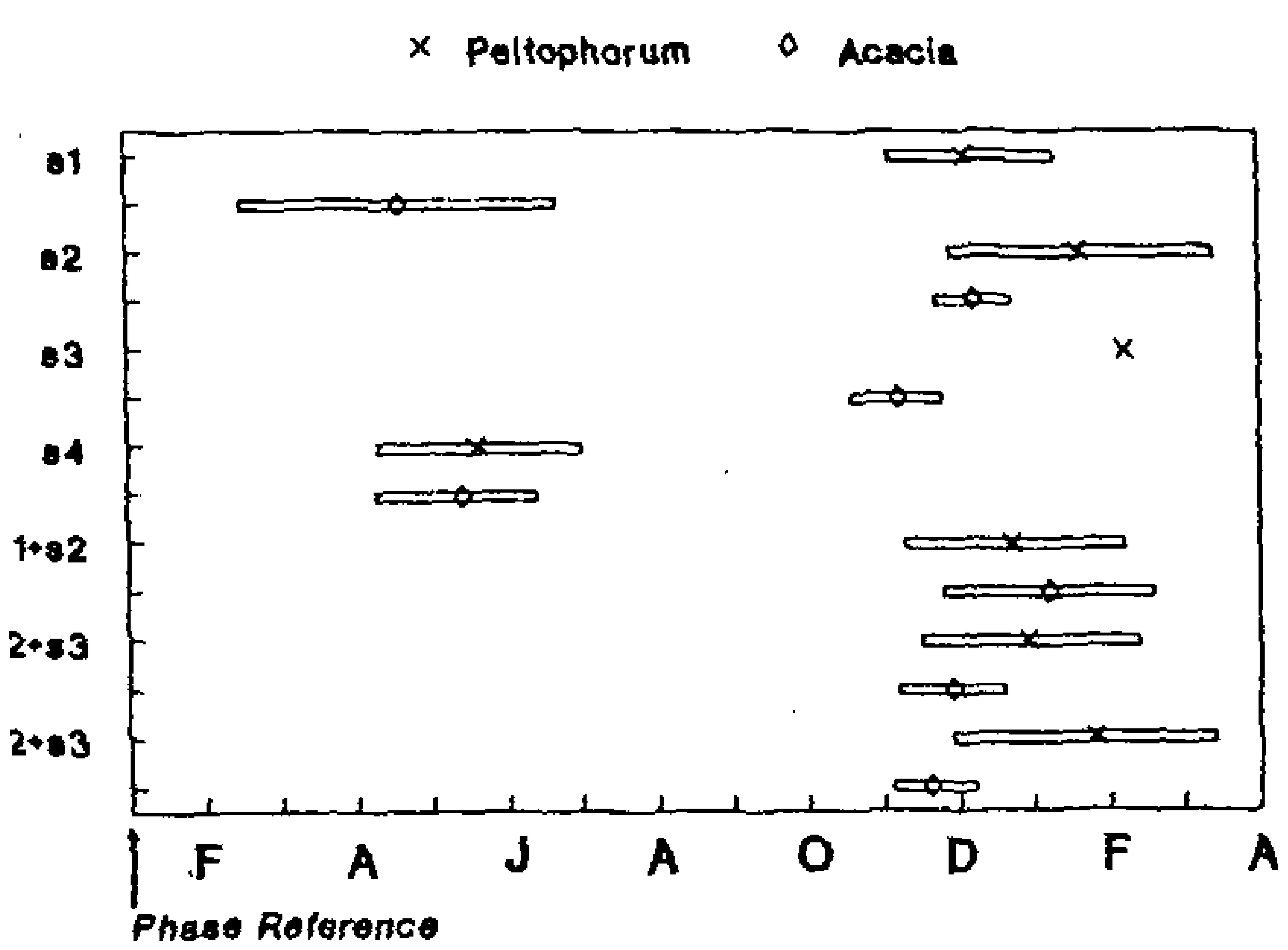
Infestation characteristics**	Main effect	
	Species (df 1.11)	Month (df 11.11)
I_1	0.067^{NS}	1.6^{NS}
I_2	26.333^{c}	2.667^{NS}
I_3	15.444^{b}	0.889^{NS}
I_4	7.0^{a}	16.0^{c}
I_t	25.869^{c}	2.087^{NS}

*ANOVA based on data shown in Table 1. Since only one score was obtained for each level of infestation in each month, species × months interaction mean square has been used as the error mean square.
**Computed as the ratio of number of plants in a given level of infestation and total number of plants.
NS = Not significant; $^{a}P \leq 0.05$; $^{b}P \leq 0.01$; $^{c}P \leq 0.001$.

Table 3. Cosinor summary showing annual rhythm in infestation intensity in *Acacia nilotica* and *Peltophorum pterocarpum*. Rhythmometry is based on 3-item moving average fitted data shown in Figure 1

Key[1]	P^2	Mesor ± SE[3]	Amplitude (95% CL)[4]	Acrophase (95% CL)[5]
Acacia nilotica				
S_1	0.003	75.0 ± 5.6	26.1 (02.8, 49.5)	Apr. 18 (Feb. 15, July 21)
S_2	< 0.001	141.3 ± 3.3	50.4 (36.8, 64.0)	Dec. 06 (Nov. 21, Dec 21)
S_3	< 0.001	132.7 ± 4.7	66.7 (47.5, 86.0)	Nov. 06 (Oct. 21, Nov.24)
S_4	0.001	29.9 ± 5.3	41.1 (19.3, 63.0)	May 12 (Apr. 09, Jun. 12)
S_1+S_2	0.005	216.3 ± 6.0	37.6 (12.7, 62.6)	Jan. 06 (Nov. 24, Feb. 18)
$S_1+S_2+S_3$	< 0.001	349.1 ± 7.5	92.0 (61.0, 123.1)	Nov. 27 (Nov. 07, Dec. 18)
S_2+S_3	< 0.001	274.0 ± 7.7	113.6 (81.9, 145.3)	Nov. 18 (Nov. 03, Dec. 06)
Peltophorum pterocarpum				
S_1	0.001	113.9 ± 3.3	24.8 (11.0, 38.5)	Dec. 03 (Nov. 03, Jan. 09)
S_2	0.021	107.8 ± 3.6	18.3 (03.6, 33.0)	Jan. 18 (Nov. 27, Mar. 12)
S_3	0.106	78.5 ± 1.4	05.0	Feb. 06
S_4	0.005	17.3 ± 4.0	25.5 (08.9, 42.1)	May 18 (Apr. 09, Jun. 27)
S_1+S_2	0.007	221.7 ± 6.7	39.9 (12.1, 67.8)	Dec. 24 (Nov. 18, Feb. 06)
$S_1+S_2+S_3$	0.007	300.2 ± 7.4	43.7 (13.3, 74.1)	Dec. 27 (Nov. 15, Feb. 12)
S_2+S_3	0.013	186.3 ± 4.3	23.1 (05.3, 40.9)	Jan. 24 (Nov. 27, Mar. 12)

[1]Infestation level (S_0 = nil; S_1 = low; S_2 = medium; S_3 = high; S_4 = very high).
[2]From F test of null amplitude rejection hypothesis.
[3]Rhythm adjusted mean of best-fitting cosine function ± 1 standard error.
[4]Half of the difference between maximum and minimum of best-fitting cosine function with 95% confidence limit.
[5]Time of maximum (in month and days) in best-fitting cosine function with 95% confidence limit.



ıre 2. Peak map. Each point represents the peak in the levels of station over an annual time scale with December 31 as the phase rence. Both ends of the horizontal bar define the range of the › confidence limit of the peak infestation. The absence of bar cates nondetection of statistically significant annual rhythm.

status of a serious pest of *A. falcataria*, a fast wing tree species introduced from Mohccas[15]. This ;gests that the bagworms are notoriously polyphagus l have the capability to switch over to a new host. :an cause heavy defoliation in various plantation sites. · *P. plagiophleps*, Nair *et al.*[8] reported that it is likely become a limiting factor for successful raising of *A. :ataria* plantations in Kerala. In addition, *Eumeta meri*, *Metisa palma* and *Brachycyttarus subteralbatus*, h of them alone or together, have been documented pests of important tree species, such as *Delonix regia*, *Tectona grandis*, *Manatha albipes*, *Terminalia catappa*, *T. indica* and *Casuarina equisetifolia*.

The studies on the dynamics of seasonal infestation caused by *E. crameri* of avenue plantations of *P. pterocarpum* and *A. nilotica* reveal that in both cases maximum outbreak is restricted to the winter months. The maximum infestation of *Peltophorum* is in December with a spread between beginning of November and mid February. However, in *Acacia* the highest infestation was noticed in November with a spread between beginning of November and the third quarter of December. Although these observations suggest that the bagworms reproduce only once in a year, the actual observations on life cycle of *E. crameri* indicate that it breeds at least 3 times in a year notably in July, October and January. It is not known why 3-item moving average fitted time series did not reveal all the generations in the form of peaks in infestation intensity. Similar kind of masking has also been reported for infestation of *P. plagiophleps* in *A. falcataria* plantations[7]. It could be reasonable to imagine that there might be some unknown natural limiting factors behind this phenomenon of masking. The pest management could become effective only when the principles which govern outbreaks of infestation are known.

This study assumes a lot of significance from regional context specially because it documents infestation profile in *A. nilotica* plantations. This plant is a common sight in the rice fields of the Chhattishgarh and being a leguminous plant it fixes atmospheric nitrogen and its compound leaves with small pinna do not obstruct the sun rays. This plant also has great economic value.

It is being used as fuel, charcoal, timber for agricultural implements and also carpentry[16]. The farmers of this region use its timber in making cart wheel and plough. In addition, it is used to make pulp in paper and rayon industries and its bark in fermentation technology for manufacturing country liquor[17]. It has also been shown to be of great value in the management of rain water in alkali soils[18].

In conclusion, the bagworm appears to be of great economic significance and therefore, a nation wide operation is desirable with a view to documenting the species and their hosts from all the corners of the country.

1. Richards, O. W. and Davies, R. G., *Textbook of Entomology*, 10th edn, Chapman and Hall, London, 1977.
2. Mathew, G. and Nair, K. S. S., Proceedings of III Oriental Entomological Symposium, 1986, vol. 2, pp. 163–167.
3. Leonhardt, B. A., Neal, J. R. Jr., Klun, J. A., Schwarz, M. and Plimmer, J. R., *Science*, 1983, **219**, 314–316.
4. Pillai, S. R. M. and Gopi, K. C., *Indian For.*, 1990, **116**, 581–583.
5. Gara, R. I., Sarango, A. and Cannon, P. G., *J. Trop. For. Sci.*, 1990, **3**, 181–186.
6. Howlader, M. A., *Bangladesh J. Zool.*, 1992, **20**, 177–183.
7. Nair, K. S. S. and Mathew, G., *Entomon*, 1992, **17**, 1–13.
8. Nair, K. S. S., Mathew, G. and Sivarajan, M., *Entomon*, 1981, **6**, 179–180.
9. Mathew, G. and Nair, K. S. S., *Indian J. For.*, 1983, **6**, 240–241.
0. Agrawal, A. and Pati, A. K., *J. Biosci.*, 1995, **20**, 409–416.
1. Nelson, W., Tong, Y., Leed, J. K. and Halberg, F., *Chronobiologia*, 1979, **6**, 305–323.
2. Gupta, S. and Pati, A. K., *J. Parisit. Appl. Anim. Biol.*, 1992, **1**, 151–163.
3. Khan, I. A. and Khanum, A., *Fundamentals of Biostatistics*, Ukaaz Publications, Hyderabad, 1994.
4. Snedecor, G. W. and Cochran, W. G., *Statistical Methods*, Iowa State University Press, Iowa, 1994.
5. *Pulping and Papermaking Properties of Fast-growing Plantation Wood Species*, FAO, Rome, 1975, pp. 466.
6. Jena, S. C., Ph D thesis, Pt Ravishankar Shukla University, Raipur, 1991.
7. Shetty, H. R., *Indian For.*, 1984, **110**, 586–590.
8. Grewal, S. S., Abrol, I. P. and Singh, O. P., *Agri. Sci.*, 1987, **57**, 30–37.

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Analysis of gene expression during embryonic development in mulberry silkworm *Bombyx mori*

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We have developed a method for dechorionation and devitellinization of the silkworm eggs without damage, to facilitate the analysis of gene expression during embryonic development of *Bombyx mori*. Making use of antibodies available from heterologous systems, the spatio-temporal expression patterns of *peroxidase* and *proliferating cell nuclear antigen* have been directly visualized in whole mount embryos at various stages of development without the need for generating transformed lines carrying specific reporter constructs. The *B. mori* system, previously unamenable for such studies, could thus serve as an attractive model for molecular analysis of insect development.

THE attention lavished on *Drosophila melanogaster* as the insect species *par excellence* for the genetic and molecular analysis of development, has eclipsed the earlier investigations on most other insects for a multitude of challenging biological phenomena. The mulberry silkworm, *Bombyx mori* has a genetic legacy comparable to that of *Drosophila*, but most of the efforts on the silkworm were channelled towards the improvement of the race through breeding, due to its economic importance[1,2].

The extended period of embryonic development of *B. mori* (10 days as compared to 24 h in *Drosophila* and 100 h in *Manduca sexta*, another widely studied Lepidopteran insect) provides an advantage to discretely analyse without overlap, the events leading to pattern formation. The early investigations on *B. mori* were confined to the problems of diapause[3] and storage of embryos or towards the elucidation of morphological landmarks by electron microscopy[3–5] and fluorescent vital dyes[6]. Recently the fate mapping of *B. mori* embryo has been carried out following laser irradiation[7]. The investigations on gene expression during early embryonic development of silkworm have lagged behind because the dechorionation and devitellinization of embryos proved to be major hurdles. Besides, the embryonic development could not be examined due to the nonavailability of cell type-specific molecular markers as well as the lack of transgenic methodologies. In such situations the immunocytochemical approaches provide an alternative. Antibodies against gene products can be exploited as tissue- and cell-specific markers to analyse phenotypes in mutant embryos[8–15]. Recent studies[8–17] have implicated the evolutionary conservation, based on sequence similarity of developmentally important genes, in a range