

## A quick and non-destructive population estimate for the weaver ant *Oecophylla smaragdina* Fab. (Hymenoptera: Formicidae)

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Field studies were made to develop a quick, non-destructive and investigator-friendly sampling plan to estimate number and biomass of *Oecophylla smaragdina* using brood nest as index in a guava ecosystem. The brood nest parameters, viz. length and breadth of each nest were measured and the number of leaves, ants outside, worker ants, winged ants, eggs, grubs and pupae was counted and subjected to correlation analyses. Based on the correlation matrix, parameters such as number of ants outside the nest, length and breadth of the nest and number of leaves in a nest were selected as independent variables and regression models were worked out to estimate biomass of *O. smaragdina* nest. These linear and curvilinear relationships (linear  $y = a + bx$ ; logarithmic  $y = C \ln x + b$ ; exponential  $y = Ce^{bx}$ ; power  $y = Cx^b$ ; polynomial  $y = b + C_{1x} + C_{2x} + C_{3x} + \dots + C_{nx}$ ) are discussed in detail as these weaver ants have several implications in ecology, biodiversity and pest management as potential biocontrol agents.

**Keywords:** Brood nest, guava ecosystem, population estimate, *Oecophylla smaragdina*, weaver ant.

ANTS are eusocial, known for their highly organized nests and may constitute up to 15–25% of the total terrestrial animal biomass<sup>1</sup>. Ants are important components of ecosystems with high ecological value and function as ‘ecosystem engineers’ – they recycle dead plants and animals, enrich the soil, pollinate flowers, spread seeds and are a major food source for many animals. They are omnivores and play an important role in linking the food web<sup>2</sup>.

The red ant, *Oecophylla smaragdina* Fab. (Hymenoptera: Formicidae) is also known as the weaver ant, green ant, Kerengga red ant, emerald leaf dweller or Indian tree ant. It is distributed in the tropical Old World from India to Taiwan and across Southeast Asia to Australia. This ant nest is arboreal, seen on trees or shrubs and made of living leaves. The workers construct the nest by gluing the edges of adjacent leaves using the sticky silk secreted by the larvae.

*Oecophylla* ants also feed on nectaries and insects. In fact, the latter aspect has been known since ages. Records show that in China, *Oecophylla* nests were introduced in

citrus orchards to control the pests of citrus since AD 300, and from then on are being utilized for biological control in fruit plantations in Australia and Asia<sup>3–5</sup>. Recently, it has been shown that introducing *Oecophylla* ants in cashew reduces the menace of the tea mosquito bug, *Helopeltis antonii* (Hemiptera: Miridae)<sup>6</sup>. Keeping this in view, methods of boosting *Oecophylla* nests in orchard plantations is gaining momentum in crops like cashew, mango, citrus, coffee, cocoa and coconut<sup>7,8</sup>. Apart from this, *Oecophylla*, a tree-dwelling and ground-foraging ant combined with other ant species contributes to the eco-dynamics of perennial tree ecosystems. In order to evaluate their roles, estimation of population numbers is important. Visual counting of ants which are found on the ground, tree trunks and in the nest is painstakingly time-consuming and fraught with painful bites. However, estimates are vital if nests have to be used or transferred, or if their role in the ecosystem is to be evaluated. Therefore, estimating the biomass of primary and secondary consumers can indicate the interspecific trophic interactions that are vital in plant–predator–prey associations and has immense ecological value. Further, it is opined that reliable estimates of *O. smaragdina* on a long-term scale will enable the use of this species as a good indicator of the well-being of ecosystem<sup>9</sup>.

Total estimation of the ant number in and around a nest, including immature and winged stages is difficult, as only a part of the foraging workers are seen outside. A few older ants which are no longer able to tend the brood, position themselves at the nest perimeter to guard the nest<sup>10</sup>. Only these guard ants and foraging ants are amenable to visual counts. It is difficult to get an estimate of the ants and brood inside the nest assuming one can gain access to the nest, as these ants are quite ferocious and can inflict a painful bite. A count of ants inside a nest is possible only by destructive sampling of the nest and killing of the ants inside. Such an approach on a long-term temporal scale is difficult and further, such enumeration by itself will be a source of population depletion. Methods for reliable estimates of ants inside the nest are inadequate<sup>11,12</sup>. A perusal of the literature shows that there is no reliable sampling method for *O. smaragdina*, especially of the ants inside the nests.

The aggressive behaviour of *O. smaragdina* and its ability to colonize extensively helps in killing or warding many pests or potential pests, notably heteropterans, foliar feeding coleopterans, lepidopterans and even vertebrates like rodents<sup>8</sup>. As mentioned earlier, this ant was found to reduce pest attack in several tree ecosystems like coconut<sup>13,14</sup>, oil palm<sup>8</sup>, cocoa<sup>15,16</sup>, citrus<sup>17</sup>, eucalyptus<sup>18</sup> and mango<sup>8</sup>. As a prerequisite to study the role of *O. smaragdina* in a guava ecosystem, it was felt that a quick, non-destructive and enumerator-friendly method to estimate both ant number and biomass in a nest (and hence the ecosystem) was necessary. Therefore, the objective of the present study was to develop such a sampling method.

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**Table 1.** Correlation matrix of different variables of ant nest

Variable	Ant number (outside)	Length of nest	Breadth of nest	Number of leaves	Number of eggs	Number of maggots	Number of pupae	Number of workers	Number of winged ants	Number of queens	Total number of ants	Weight of ants
Ant number (outside nest)	1.00											
Length of nest	0.74*	1.00										
Breadth of nest	0.77*	0.87*	1.00									
Number of leaves	0.97*	0.77*	0.78*	1.00								
Number of eggs	0.84*	0.69*	0.75*	0.79*	1.00							
Number of maggots	0.88*	0.68*	0.79*	0.82*	0.89*	1.00						
Number of pupae	0.84*	0.54*	0.49*	0.86*	0.52*	0.60*	1.00					
Number of workers	0.94*	0.66*	0.75*	0.93*	0.80*	0.90*	0.87*	1.00				
Number of winged ants	0.86*	0.61*	0.65*	0.89*	0.68*	0.66*	0.76*	0.80*	1.00			
Number of queens	-0.12	-0.24	-0.24	-0.15	-0.10	-0.10	-0.09	-0.14	-0.10	1.00		
Total number of ants	0.97*	0.70*	0.77*	0.95*	0.85*	0.92*	0.85*	0.99*	0.83*	-0.13	1.00	
Weight of ants (g)	0.94*	0.67*	0.74*	0.94*	0.78*	0.89*	0.89*	0.99*	0.80*	-0.12	0.99*	1.00

\*Significant at  $P < 0.01\%$ .

In a guava field, the nests of *O. smaragdina* are visible and accessible on the trees. The number of leaves which make up a nest, length and breadth of the nest, and ants found foraging or guarding just around it can be estimated. These parameters serve as independent variables. It was hypothesized that these measures are related to the number and weight of ants inside a nest (including immature stages as dependable variables). If such a relationship holds good either as linear or nonlinear, the model then arrived at will help obtain estimates of the total number and weight of ants inside the nest. Therefore, this will serve as non-destructive sampling to get the ant number and biomass in a guava ecosystem.

The study was conducted in an unsprayed guava *Psidium guajava* L. (cv. *Allahabad safeda*) orchard of 4 ha at the Indian Institute of Horticultural Research, Hesaraghatta, Bangalore (12°58'N; 77°35'E). Thirty nests were randomly chosen and labelled. The ants outside each nest were counted at 9.30 a.m., 11.30 a.m. and 3.30 p.m. to see if variation existed in the number of ants with time of day. After three such counts, each nest was harvested and brought to the laboratory. Care was taken to snap above the nest using a secateur and trap the nest within a polythene bag 30 × 30 cm<sup>2</sup> to prevent escape of any ant or brood. Then the polythene bags were sealed and brought to the laboratory. The ants were anesthetized using ethyl acetate on a cotton wad. The length and breadth of each nest were measured and the number of leaves and buds was counted. Each nest was then split and the other parameters, viz. number of worker ants, winged ants (including queen), eggs, grubs and pupae was counted. Then the total number and fresh weight of all the ant forms (eggs + grubs + pupae + winged ants + queen) from each nest were recorded.

The data of ants counted outside the nests at three different times during the day (viz. 9.30 a.m., 11.30 a.m. and 3.30 p.m.) were subjected to ANOVA, to see if there were differences in the counts. LSD ( $P = 0.05$ ) was used

as a test criterion<sup>19</sup>. As there was no significant difference in the data for the three timings, the number of ants found outside at one of the timings, viz. 11.30 a.m. was used for further analyses. The data on the count of ants found outside and various parameters were subjected to correlation analyses and the matrix is presented in Table 1. The parameters – length of nest, breadth of nest, number of leaves (taken as independent variables), number of immature stages, total number and weight of all ants (dependent variables) were subjected to linear and curvilinear regression analyses to arrive at prediction models using a scatter diagram.  $R^2$  explained the variability of the dependent variables due to an independent variable. Applicability of regression analysis to biological studies is well known as it explores the degree of relationship in the pattern of variation of two or more variables with coefficient of determination  $R^2$  (which is a measure of how well the variation of one variable explains the variation of the other) as the criterion<sup>19,20</sup>.

The study of variations in ant movement with time of the day revealed no significant difference in ant movement outside the nest for the three timings (viz. 9.30 a.m., 11.30 a.m. and 3.30 p.m.). The means (ants/nest) of the foraging ants outside the nest ( $n = 30$ ) at the three timings were  $27.87 \pm 6.81$  ( $\sigma = 37.28$ ),  $39.47 \pm 7.77$  ( $\sigma = 42.53$ ) and  $28.3 \pm 14.46$  ( $\sigma = 79.20$ ) respectively. There were no significant differences among these means at LCD ( $P = 0.05$ ). The results of the correlation matrix are tabulated in Table 1. The length and breadth of the nests and number of leaves showed highly significant correlation with the number of eggs, grubs, pupae, workers, winged ants, total ants and total weight of ants. Among the three parameters, the number of leaves showed higher correlation coefficients, especially for the total number of ants and weight.

Based on the correlation matrix, parameters such as number of ants outside the nest, length and breadth of a nest and the number of leaves in a nest were selected as

**Table 2.** Models with number of ants as independent variable

Dependent variable	Model	Equation	$R^2$
Number of eggs	Linear	$y = 6.35x - 19.62$	0.71
	Polynomial	$y = -0.01x^2 + 7.96x - 43.1$	0.71
Number of grubs	Linear	$y = 14.77x - 60.71$	0.77
	Polynomial	$y = -0.05x^2 + 29.11x - 269.41$	0.82
Number of pupae	Linear	$y = 9.72x - 61.11$	0.70
	Polynomial	$y = -0.01x^2 + 14.02x - 123.67$	0.71
Number of workers	Linear	$y = 23.72x + 240$	0.88
	Polynomial	$y = -0.08x^2 + 48.91x - 126.59$	0.95
Total number of ants	Linear	$y = 0.02x + 1.41$	0.94
	Logarithmic	$y = 31.93\ln(x) - 165.52$	0.49
	Polynomial	$y = -0.13x^2 + 99.73x - 502.52$	0.96
Total weight of ants (g)	Linear	$y = 2.52x + 2.85$	0.88
	Logarithmic	$y = 28.39\ln(x) + 9.86$	0.47
	Polynomial	$y = -0.001x^2 + 0.71x - 4.50$	0.95

**Table 3.** Models with length of nest as independent variable

Dependent variable	Model	Equation	$R^2$
Number of eggs	Linear	$y = 73.60x - 779.72$	0.47
	Logarithmic	$y = 1062.8\ln(x) - 2481.8$	0.41
	Polynomial	$y = 5.36x^2 - 111.28x + 586.54$	0.54
Number of grubs	Linear	$y = 160.49x - 1681.5$	0.46
	Logarithmic	$y = 2278\ln(x) - 5292.3$	0.38
	Polynomial	$y = 14.33x^2 - 333.69x + 1970.3$	0.55
Number of pupae	Linear	$y = 88.09x - 886.93$	0.29
	Logarithmic	$y = 1243.3\ln(x) - 227.58x + 1445.9$	0.24
	Polynomial	$y = 9.15x^2 - 227.58x + 1445.9$	0.37
Number of workers	Linear	$y = 237.33x - 2082.6$	0.44
	Logarithmic	$y = 3416.3\ln(x) - 7543.7$	0.38
	Polynomial	$y = 19.04x^2 - 419.43x + 2770.7$	0.52
	Power	$y = 0.83x^{2.43}$	0.32
	Exponential	$y = 50.62e^{0.15x}$	0.31
Total number of ants	Linear	$y = 619.88x - 6054.7$	0.49
	Logarithmic	$y = 8832.8\ln(x) - 20088$	0.41
	Polynomial	$y = 56.40x^2 - 1325.7x + 8322.5$	0.60
	Power	$y = 0.2201x^{3.29}$	0.38
	Exponential	$y = 0.1532e^{0.21x}$	0.39
Total weight of ants (g)	Linear	$y = 3.54x - 34.07$	0.45
	Logarithmic	$y = 50.66\ln(x) - 114.75$	0.38
	Polynomial	$y = 0.3x^2 - 6.81x + 42.39$	0.54
	Power	$y = 0.001x^{3.29}$	0.38
	Exponential	$y = 0.15e^{0.21x}$	0.39

independent variables, and models (linear and curvilinear) were worked out (Tables 2–5). Usually the linear regression assumes that the data fit to a straight line which is not common in many biological studies. In such cases a data transformation may help or it is necessary to use polynomial regression or other curvilinear relationships, viz. logarithmic, power, exponential and polynomial<sup>19</sup>.

The number of ants outside the nest (NO) as a parameter could explain variability in egg and pupal numbers to the extent of 70–71% based on  $R^2$  of linear and polynomial (order 2) models respectively. In case of grubs, NO could

explain the variability in grubs and workers inside the nest from 77% to 82% and 88% to 95% respectively, for linear and polynomial (order 2) models. However, NO with linear equation could explain variability in the total number of ants to the extent of 94% ( $R^2 = 0.94$ ). With polynomial (order 2),  $R^2$  increased to 96%. For total weight of ants,  $R^2$  was 88% and 95% respectively, for linear and polynomial (order 2) models (Table 2).

The length of the nest (LN) as an independent variable did not explain the variability adequately in the number of eggs, grubs, pupae and workers, as evident from Table 2.

**Table 4.** Models with breadth of nest as independent variable

Dependent variable	Model	Equation	R <sup>2</sup>
Number of eggs	Linear	$y = 98.3x - 522.76$	0.56
	Logarithmic	$y = 755.36\ln(x) - 1208.5$	0.40
	Polynomial	$y = 7.06x^2 - 62.52x + 178.35$	0.66
Number of grubs	Linear	$y = 232.86x - 1263.3$	0.63
	Logarithmic	$y = 1747\ln(x) - 2806.9$	0.43
	Polynomial	$y = 19.11x^2 - 210.64x + 634.89$	0.79
Number of pupae	Linear	$y = 98.82x - 435.02$	0.24
	Logarithmic	$y = 878.48\ln(x) - 1351.3$	0.23
	Polynomial	$y = -4.03x^2 + 192.37x - 835.41$	0.25
Number of workers	Linear	$y = 329.18x - 1347.8$	0.56
	Logarithmic	$y = 2716.8\ln(x) - 4000.8$	0.46
	Polynomial	$y = 9.29x^2 + 113.61x - 425.17$	0.57
	Power	$y = 7.27x^{2.13}$	0.47
	Exponential	$y = 77.65e^{0.22x}$	0.42
Total number of ants	Linear	$y = 837.87x - 3967.3$	0.59
	Logarithmic	$y = 6729.8\ln(x) - 10367$	0.46
	Polynomial	$y = -33.98x^2 + 48.95x - 590.84$	0.62
	Power	$y = 4.92x^{2.53}$	0.38
	Exponential	$y = 80.37e^{0.27x}$	0.47
Total weight of ants (g)	Linear	$y = 4.80x - 22.23$	0.54
	Logarithmic	$y = 39.47\ln(x) - 60.67$	0.44
	Polynomial	$y = -0.13x^2 + 1.85x - 9.61$	0.55
	Power	$y = 0.01x^{2.92}$	0.58
	Exponential	$y = 0.28e^{0.30x}$	0.52

**Table 5.** Models with number of leaves in a nest as independent variable

Dependent variable	Model	Equation	R <sup>2</sup>
Number of eggs	Linear	$y = 16.82x - 110.62$	0.62
	Logarithmic	$y = 411.98\ln(x) - 787.13$	0.44
	Polynomial	$y = -0.004x^2 + 17.63x - 115.19$	0.62
Number of grubs	Linear	$y = 38.83x - 266.34$	0.67
	Logarithmic	$y = 949.87\ln(x) - 1825$	0.47
	Polynomial	$y = -0.19x^2 + 60.45x - 486.21$	0.69
Number of pupae	Linear	$y = 28.33x - 252.54$	0.75
	Logarithmic	$y = 686.17\ln(x) - 1372.8$	0.52
	Polynomial	$y = -0.01x^2 + 27.14x - 240.39$	0.75
Number of workers	Linear	$y = 66.22x - 168.28$	0.86
	Logarithmic	$y = 1713.11\ln(x) - 3056.9$	0.68
	Polynomial	$y = -0.33x^2 + 104.202x - 552.86$	0.89
	Power	$y = 20.96x^{1.21}$	0.57
	Exponential	$y = 193.85e^{0.04x}$	0.48
Total number of ants	Linear	$y = 167.62x - 946.38$	0.90
	Logarithmic	$y = 4165.8\ln(x) - 7836.8$	0.65
	Polynomial	$y = -0.40x^2 + 213.71x - 1415.3$	0.91
	Power	$y = 17.97x^{1.43}$	0.62
	Exponential	$y = 244.87e^{0.05x}$	0.53
Total weight of ants (g)	Linear	$y = 0.99x - 5.50$	0.87
	Logarithmic	$y = 25.44\ln(x) - 48.33$	0.68
	Polynomial	$y = -0.005x^2 + 1.54x - 11.09$	0.90
	Power	$y = 0.04x^{1.71}$	0.73
	Exponential	$y = 1.02e^{0.05x}$	0.55

All the R<sup>2</sup> values were low, ranging from 0.24 to 0.55. Further, in different models worked out for LN as an independent variable did not account appreciably for the variability in the total number of ants and total weight of ants. The R<sup>2</sup> values ranged from 0.38 to 0.60 for total

number of ants. For total weight of ants as dependent variable, R<sup>2</sup> ranged from 0.38 to 0.54 (Table 3).

The breadth of the nest (BN) as an independent variable, did not appreciably account for the variability in the number of eggs, grubs, pupae and workers, except in the

case of a polynomial (order 2) for grubs, where  $R^2$  was 0.79 (Table 4). Further as in LN, the different models with BN as an independent variable could explain variability from 38% to 62% and 44% to 58% respectively, in the total number and weight of ants (Table 4).

Of all the regression models tried, linear and polynomial (order 2) showed high  $R^2$  values of 0.86 and 0.89 respectively, when the number of workers ( $x$ ) and the number of leaves in a nest (NL) ( $y$ ) were regressed (Table 5). However, with respect to eggs, grubs and pupae, all the models worked out had  $R^2$  values  $<0.75$  (Table 5). Through linear and polynomial models, NL could explain the variability in the total number and weight of ants to the extent of 90%, 91%, 87% and 90% ( $R^2$  values) respectively (Table 5). Whereas the other models worked out had lesser  $R^2$  values.

Ants contribute significantly to biodiversity and often are an important index of species richness. To assess the importance of ants, estimation of the population is essential. Nest-dwelling ants like *O. smaragdina* are not easily amenable to visual *in situ* counts, especially where good number of workers, queen and immature stages are inside the nests. Those that are visible outside the nests constitute a small proportion of the total ant numbers of a nest, and are represented mainly by the older workers, which guard the nest, and a segment of the foragers<sup>10</sup>. Only these ants are amenable to visual counts. The risk of foragers intermingling from other colonies can be ruled out in ants. As in typical formicids, the colony odour can function as separators, preventing overlap of inter-colony foragers<sup>21</sup>. These ants are perhaps representatives of the colony size present. So, in the present study, the number of ants outside was hypothesized to be a useful index to estimate the total number of ants and their biomass in a nest. Further, this will obviate the necessity of harvesting each nest, killing the ants and then counting their numbers. Harvesting the nest is invariable fraught with irritating bites which *O. smaragdina* can inflict, as this species is aggressive and ferocious towards any intruders.

The study, considering the number of ants outside as independent variable, showed that polynomial order 2 and linear models with  $R^2 = 0.96$  and  $0.94$  were adequate to explain the variability in the total number of ants in a nest (Table 2). These models hold when ant nests are sparse on a tree canopy and the ants outside can be enumerated with ease. It was found that these models were also adequate for estimating total weight of ants. The linear and polynomial models showed high  $R^2$  for estimating the number of workers inside a nest, when the number of ants outside was used as an independent variable. This is crucial as workers inside the nest are vital in the sustenance of the colony. They take care of the brood, and as they age, forage for food and guard the nest<sup>10</sup>. But the number of ants outside is not a potential predictor of egg number, though the polynomial model for grubs had  $R^2 = 0.82$ .

However, *in situ* visual counting of the ants outside is feasible on a guava tree, only if the canopy is low and the models are reliable if the estimator is sure that intermingling between nests can be ruled out. If there are many nests or the canopy is beyond a normal visual range of the estimator, then counting of ants outside the nest is difficult. In such cases one has to resort to other visible parameters, viz. length, breadth or number of leaves in the nest.

The independent variables, viz. length and breadth of the nest were not reliable in the estimation of different stages of the ants or total number/weight of ants inside the nest. However, the number of leaves used in nest-building as an independent variable by far was found to be the best parameter in obtaining estimates of total number and weight of ants based on the high  $R^2$  values (Table 5). Thus, the number of guava leaves in the construction of a nest by *O. smaragdina* was found to be a dependable criterion and can be used to arrive at total number of ants in each nest.

The number of leaves in a nest showed a stronger linear relationship with the total weight of ants in a nest ( $R^2 = 0.87$ ). So the number of leaves can be used to estimate the total number and weight (biomass) of ants in a nest. It may be mentioned that using a pair of binoculars (7× or 8×) makes counting the number of leaves in a nest easier. Estimation of total number and weight of ants in a whole guava ecosystem with reference to *O. smaragdina* is basic to understand the exact role of these ants in the community structure of arthropods of the guava ecosystem. In a Brazilian tropical forest, ants have been estimated to have a biomass four times that of all vertebrates put together<sup>17</sup>. From a biodiversity point of view, there is a kind of lopsidedness, as an overwhelming biomass of living organisms is contributed by insects, in which ants have a significant role<sup>18</sup>. So, ants being dominant and successful components of the ecosystem would be good indicators of its well-being<sup>7</sup>.

In the present study the model developed with the number of leaves as the independent variable was found to have a high precision and was useful in quick sampling of ant number and weight. When done periodically on a temporal scale, the population dynamics of the ants can be worked out. Further, using the number of leaves as an independent variable is convenient and reliable, especially when several nests on a tree occur interconnected, and assigning the ants outside to a particular nest becomes difficult. Other variables like the length and breadth of the nest did not show strong relationship with the total number and weight of ants present in a nest. Even if they did, accurate measuring of the nest without harvesting it is difficult. Considering all these factors, counting the number of leaves used in nest construction is less cumbersome, and for a nest which is high in the canopy, a pair of binoculars could be used. In the guava ecosystem, where trees are generally not beyond 4 m, the sampling

models outlined here are feasible and viable. Previous studies used nest length and breadth as criteria to develop models for population estimation of weaver ants<sup>20</sup>. However, the present study has proved that a simple model using the number of leaves involved in nest construction provides better estimates of ant population compared to any other model. Further, as *O. smaragdina* is a vigilant and territorial predator of many insect pests in a number of horticultural crops, population estimation through simple models as indicated in the present study will help in designing colony relocation studies to use them more efficiently in arboreal domains.

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## Moth pests collected in light traps of tea plantations in North East India: species composition, seasonality and effect of habitat type

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Light trap has often been used in the ecological studies of lepidopteran insect pests in agroecosystems. However, the light trap in Indian agricultural systems is rarely adopted either to monitor the population size or to study the migration of moth pests. In the present study, we have installed light traps in shaded and unshaded tea plantations of North East (NE) India to study (1) the species composition, (2) effect of shade on moth pests, (3) seasonality of major pests and (4) to learn the sex proportion of major pests captured in light traps. The two-year catches in light traps suggested that *Hyposidra talaca* (Geometridae) is a major pest of tea in NE India. It peaks in number during winter months, with relatively few moths caught in the later parts of the year. *Eterutia magnifera* (Zygaeni-

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