Estimation of the population density of the leaf miner, *Phytomyza ranunculi* and of the surface area of leaves of the garden rununculus, *Ranunculus asiaticus*, as its host plant.*

By TUYOSI SUGIMOTO, SUSUMU MATUMOTO and TADAHIRO MARUYAMA

Summary In the present paper it was intended to establish the sampling design to estimate the population density of the leaf mining fly, *Phytomyza ranunculi*, and the surface area of leaves of the garden ranunculus, *Ranunculus asiaticus*, as its host plant.

The insect population distributed spacially in the overdispersed pattern in all stages of egg and larva. So the original counts (x) should be transformed to $\sin h^{-1}\sqrt{\frac{\beta-1}{\alpha-1}}x$ before the analysis of variance. It was recognized that the number of insects per leaf varied significantly between branch types but did not between plants. On the other hand, the surface area of leaf of host plant per leaf differed significantly both between branch types and between plants. To know the variation of the number of leaves per plant, the number of branches on a plant and the number of leaves per branch were separately subjected to the analysis. The result showed that there were no significant differences between branch types and between plants. Also because there was no significant correlation between the number of leaves per plant and the number of insects per leaf or the surface area of leaf per leaf, two values in each pair seem to be independent of each other. The significant correlation, however, was recognized between the number of larvae per leaf and the surface area of leaf per leaf.

From above analyses the population should be stratified basing upon the branch types so as to obtain the more efficient estimates of the number of insects per plant, of the surface area of leaf per plant and of the number of insects per unit surface area of leaf. The last section shows the methods to estimate those values by the subsampling or two- stage sampling with primary units of unequal size.

The detailed studies on the population ecology of organisms require necessarily the precise method for estimating population parameters. This paper deals with methods for estimating the population density in the egg and the larval stages of the leaf mining fly, *Phytomyza ranunculi Schrank* and also the surface area of leaf which would play an extremely important part for the existence of the larvae because of their habitats and food resources. Especially, in the present paper it is intended to express the population density as the mean number of insects per unit surface area of leaf.

A female of *P. ranunculi* lays eggs in the leaf tissue of a host plant, classified into the *Ranunculaceae*, by inserting her ovipositor into the tissue. The larvae

* Laboratory of Entomology, Faculty of Agriculture, Kyoto Prefectural University, Kyoto, Japan. (Contribution No, 124)
Received on Aug, 31, 1969.

make the mines in the leaf as a result of feeding inner tissue of the leaf as a food resource. After they live in the leaf tissue during three instar periods in such a way, they get out of the leaf to the outer world so as to pupate.

Method

The present study was undertaken in the experimental farm in Kyoto Prefectural University from early April to middle June in 1969. In the course of this work the garden ranunculuses, *Ranunculus asiaticus Linnaenus,* which were used as a host plant, were subjected to the natural condition as much as possible. They were, however, sometimes covered with victoria lawn tents so as to protect them from blasting by the frost when it threatened to have frost in the late spring.

In this work a leaf was adopted as the least sampling unit. The so-called subsampling or two-stage sampling was undertaken to estimate the population density of insect and the surface area of leaf. That is to say, in the first step ten plants were selected at random from one hundred plants in the field and in the next step the five leaves were removed at random from each group of the branch included in each of ten plants which was classified basing upon the branch type, i. e. a single branch and a branch consisted of more than two twigs. Also the number of branches on the selected plants and the number of leaves on the five branches selected from each of those plants were counted in each branch type.

So as to count the number of eggs in a leaf the eggs in the leaves were stained in the saturated solution of the scarlet red in 70% alcohl (Yokoo, 1959) which was devised to examine the number of nematodes in the plant tissue (in press).

The descriminant function method (Sugimoto, 1967) was used so as to determine the instar of larvae in a leaf. The body length of larva and the width of mine which were adopted as variates in the function were measured by a binocular microscope.

The surface area of leaf was measured from leaf to leaf by the automatic area meter.

Results and Discussion

Spacial distribution pattern

So as to analyze the raw data from the ecological field experiment it is generally important to clarify the spacial distribution pattern of the organism concerned. Iwao (1968) found there was a linear regression, $\overset{*}{m}=\alpha+\beta m$, between the mean crowding ($\overset{*}{m}$) (Lloyd, 1967) and the mean density (m) in many insect populations regardless of their specific patterns of spacial distribution. Also he pointed out that the values of two constants α and β in the regression function depend on the distribution pattern. So as to clarify the spacial distribution in the present species the values of $\overset{*}{m}$ and m were calculated in each developmental stage from the data including both living and dead individuals. The figure 1 shows the linear relations between $\overset{*}{m}$ and m with the values of α and β in the regression functions. Because

$\alpha>0$ and $\beta>0$ in all developmental stages in the figure 1, the present species seems to distribute in the overdispersed pattern including the negative binomial distribution regardless of the developmental stages. This result does not, however, necessarily mean that the present species itself distributes in the overdispersed pattern because the surface area of a leaf which was adopted as a sampling unit varies from leaf to leaf.

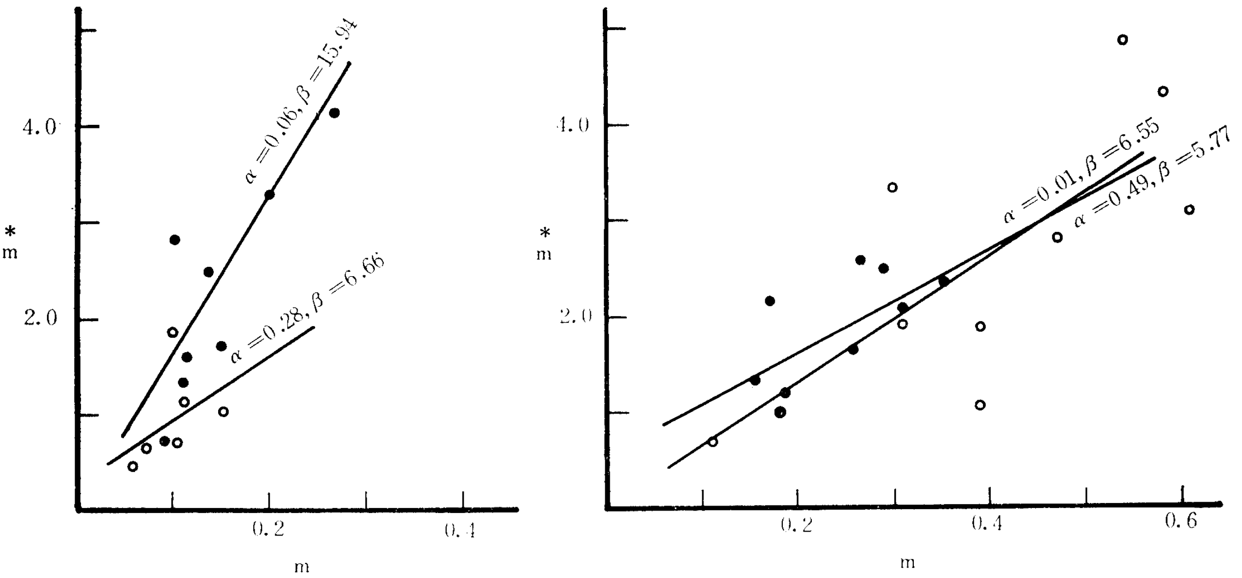

Fig. 1. Relations of mean crowding ($\overset{*}{m}$) to mean density (m) in four developmental stages. The solid and open circles represent the relations in the egg and the first instar larva in the left figure and also in the second and the third instar larva in the right one, respectively.

Transformation of original counts

In the analysis of variance the variance is assumed to be independent of the mean. If the variance relates to the mean in some way, the original count should be transformed to the value which can stabilize the variance before the analysis of variance. Recently, Iwao and Kuno (1968) proposed a variety of forms of transformation in accordance with the values of α and β in the regression of the mean crowding on the mean density mentioned above. Following their propositions the present data corresponded with the category 1 with the condition $\alpha>-1$ and $\beta>1$. So the original counts (x) were transformed to $f(x)=\sin h^{-1}\sqrt{\frac{\beta-1}{\alpha-1}x}$.

Bases for stratification

Number of eggs or larvae per leaf

The four samples taken during the late April to early May in 1969 were subjected to the analysis so as to clarify whether there were the significant differences between sampling times, between plants and between branch types and also the significant interactions between those factors. In the table 1 it is shown that there was significant difference only between sampling times in the eggs and between branch types in the second and the third instar larvae but no difference in the first instar larvae so far as those three factors are concerned. In any case the significant F value was not recognized between plants. Those results indicate that

Table 1. The analysis of variance in study of inter-and intra-plant valiability in the *P. ranunculi* population.

		Egg		1st instar larva		2nd instar larva		3rd instar larva	
	Degree of freedom	Mean square	F	Mean square	F	Mean square	F	Mean square	F
Sampling times	3	4.063	3.396**	1.477	2.424	0.562	0.926	0.313	0.455
Plants	9	1.572	1.314	0.785	1.287	1.014	1.669	1.251	1.814
Branch types	1	0.731	0.611	0.743	1.219	5.483	9.025**	9.015	13.072**
S×P	27	0.995	0.832	0.924	1.516	0.930	1.531	1.173	1.700**
S×B	3	0.219	0.182	1.154	1.895	0.372	0.613	1.117	1.619
P×B	9	2.591	2.165	0.491	0.805	0.529	0.855	0.211	0.306
S×P×B	27	2.001	1.672*	0.537	0.882	0.643	1.058	1.189	1.725*
Error	320	1.917		0.609		0.608		0.690	

* Significant within the 0.05 level
** Significant within the 0.01 level

the population should be stratified basing on the branch types to estimate the mean number of insects per leaf more precisely because a between sampling times has not significant meaning for the stratification of the population in practice.

Surface area of leaf per leaf

Prior to the analysis of variance it was assumed that the surface area of a leaf distributes with the normality and additivity of effect. In this case the analysis was made on the four samples analyzed above and also the variance was analyzed to the three factors discussed above. In the table 2 it is observed that those three factors affect significantly the variation in the surface area of leaf per leaf. So the results of analysis mean that it will be better to stratify the population in more fractions basing upon some nature of

Table 2. The analysis of variance in the study of inter-and intra-plant valiability in the surface area of leaf.

	Degree of freedom	Mean square	F
Sampling times	3	183.515	17.653**
Plants	9	42.349	4.074**
Branch types	1	701.323	67.462**
S×P	27	15.719	1.512*
S×B	3	14.700	1.414
P×B	9	6.154	0.592
S×P×B	27	8.803	0.847
Error	320	10.396	

* Significant within the 0.05 level.
** Significant within the 0.01 level.

plants in addition to branch types so as to estimate the mean surface area of leaf per leaf with better efficiency.

Number of leaves per plant

The population density of insect and the surface area of leaf had better to be expressed as the total value per plant so as to discuss the seasonal fluctuation of the values. As those estimated total values relate compactly to the number of leaves on a plant, the analysis of variance for testing the variation in the total number of leaves on a plant must be made in the first step. Unfortunately, in the present work the total number of leaves on a plant was not counted but the number of branches on the sampled plants and the number of leaves on ten branches selected from them were counted separately. So the analysis was undertaken individually to those two kinds of observation which relate compactly to the estimated total number of leaves per plant. Firstly, from the table 3 it can be concluded that any factor does not affect significantly the variation in the number of branches on a plant so far as those three factors are concerned. In this analysis there is no interaction between factors because the number of branches on a plant has no replication. Nextly, the

Table 3. The analysis of variance in the study of inter- and intra-plant valiability in the number of branches on a plant and of leaves on a branch.

	No. of branches on a plant			No. of leaves on a branch		
	Degree of freedom	Mean square	F	Degree of freedom	Mean square	F
Sampling times	3	287.283	1.229	3	1.409	1.833
Plants	9	449.866	1.925	9	1.847	2.402*
Branch types	1	312.050	1.335	1	0.003	0.001
S×P				27	1.163	1.512*
S×B				3	1.549	2.015
P×B				9	0.369	0.480
S×P×B				27	0.945	1.230
Error	66	233.742		320	0.769	

* Significant within the 0.05 level.

number of leaves on a branch, which varies within the range of one to four, differs a little significantly between plants but did not between branch types (Tab. 3). So those two results may indicate that the total number of leaves on a plant varies a little significantly only between plants. It may, however, safely be said that it would not introduce an important mistake to disregard the difference between plants because the F value is not large. If the above argument is true, it may be infered that the total number of insects per plant varies significantly only between branch types and on the other hand the total surface area of leaves per plant does significantly between plants in addition. Ives (1955) classified the trees into several categories to make the subset of data more homogeneous in his work of estimating the egg density of larch saw fly, *Pristiphora erichsonii*. On the other hand, Morris (1955) ignored the significant differences between trees in his study to estimate the population density of the spruce budworm, *Choristoneura fumiferana* because "it is more probable that they are obscured by random sampling error when only a few trees are studied". In the present study it may be better to disregard the difference between plants even so as to estimate the total surface area of leaf per plant because it is difficult and very troublesome in practice to classify the plants growing in the same degree to some categories basing on their external appearance.

Relationship of mean number of insects per leaf and mean surface area of leaf per leaf to number of leaves on a plant

The total number of insects per plant or the total surface area of leaves per plant can be obtained by multiplying the mean of the value per leaf by the number of leaves on a plant. So two values constituting the product should be statistically independent of each other. In order to examine this independency the correlation between the mean number of insects per leaf or the mean surface area of leaf per leaf and the number of leaves on a plant was discussed in each stratum from a sample drawn in early May. Here, the product of the number of branches on a plant and the mean number of leaves per branch was used in place of the number of leaves on a plant for convenience sake because the number of leaves on a plant was not examined in this work as mentioned above. In the table 4 there are

Table 4. Calculated correlation between two values in each pair of the number of insects per leaf, the surface area per leaf and the number of leaves per plant in two strata.

	Egg		Third instar larva	
	BI	BII	BI	BII
No. of insects — No. of leaves	0.099	0.359	−0.397	0.328
Surface area — No. of leaves	0.452	−0.255		
No. of insects — Surface area	0.154	−0.383	0.779**	0.781**

** Significant within the 0.01 level.
BI and BII denote the strata of a single branch and of a branch consisted of more than two twigs, respectively.

significant correlations neither between the mean number of insects per leaf and the number of leaves on a plant in the eggs and the third instar larvae nor between the surface area of leaf per leaf and the number of leaves on a plant. This indicates that the mean number of eggs or third instar larvae per leaf or the mean surface area of leaf per leaf is independent of the total number of leaves on a plant. Also in the table 4 the comperatively high correlations was found between the mean number of insects per leaf and the mean surface area of leaf per leaf in the third instar larvae. This result may be expected as a matter of course because the larger leaf would tend to be infested by the more insects. The significant correlation, however, was not recognized between the mean number of eggs per leaf and the mean surface area of leaf per leaf.

Estimation of number of insects per plant, of surface area of leaf per plant and of number of insects per unit surface area of leaf

Firstly, it is intended to estimate the mean number of insects per plant and the mean surface area of leaf per plant. It would be better to undertake the sub-sampling with primary units of unequal size so as to estimate those values because the sampling units have the double structure, i.e. plant and leaf, in the present population. This sampling method is consisted of two steps, that is to say, the first step is to select a certain number of plants at random from the population and the second is to select a certain number of leaves from each chosen plant at random. Also it would be better to stratify the population on the basis of the branch types in order to obtain more efficient estimate. In this work the probability which a certain plant is selected is assigned equally to all plants. So the estimate of the mean number of insects per plant Y is:

$$Y=\frac{1}{n}\sum_{h=1}^{n}\sum_{i=1}^{2}M_{hi}\,\bar{y}_{hi}$$

where n is the number of plants sampled from the population,

M_{hi} is the number of leaves on the ith stratum on the hth plant,

y_{hi} is the mean number of insects per leaf in the ith stratum on the hth plant.

Ignoring the finite population correction, the variance of above estimate $V(Y)$ is:

$$V(Y)=\frac{1}{n^2}\sum_{h=1}^{n}\sum_{i=1}^{2}M_{hi}^2\,s^2\bar{x}_{hi}$$

where $$s^2\bar{x}_{hi}=\frac{s^2x_{hi}}{k_{hi}}=\frac{\sum_{j=1}^{k_{hi}}(x_{hij}-\bar{x}_{hi})^2}{k_{hi}(k_{hi}-1)}$$

x_{hij} is the number of insects on the jth leaf in the ith stratum on the hth plant,

k_{hi} is the number of leaves sampled from the ith stratum on the hth plant.

In order to estimate the mean surface area of leaves per plant Y and $\bar{y}_{hi}$ in the above formulas should be replaced to the mean surface area of leaves per plant X and the mean surface area of leaf per leaf in the ith stratum on the hth plant $\bar{x}_{hi}$, respectively.

Nextly, ignoring the finite population correction, the mean number of insects per unit surface area of leaf R was estimated by the use of the combined ratio estimate (Cochran, 1953), that is to say, a method to obtain more precise estimate by taking advantage of the correlation between two variates.

$$R=\frac{\sum_{h=1}^{n}\sum_{i=1}^{2}M_{hi}\,\bar{y}_{hi}}{\sum_{h=1}^{n}\sum_{i=1}^{2}M_{hi}\,\bar{x}_{hi}}$$

$$V(R)=\frac{1}{n(n-1)X^2}\sum_{h=1}^{n}\sum_{i=1}^{2}(d_{hi}'-\bar{d}_h')^2$$

where $d'_{hi}=N\,M_{hi}\bar{d}_{hi}$, $\quad \bar{d}'_h=\frac{1}{n}\sum_{h=1}^{n}d_{hi}'$, $\quad \bar{d}_{hi}=\bar{y}_{hi}-R\bar{x}_{hi}$

X is the total surface area of leaf in the population,

N is the total number of plants in the population.

As X is not known in the present case, the sample estimate of X, $\frac{N}{n}\sum_{h=1}^{2}\sum_{i=1}^{2} M_{hi}\bar{x}_{hi}$, is substituted for it.

Acknowledgement

The authors wish to express their grateful appreciation to Prof. M. Sasakawa, Entomological Laboratory, Kyoto Prefectural University, for his kind encouragement and suggestion and also to Prof. T. Nakagiri and Mr. K. Inoue, Mathematical Laboratory, Kyoto Prefectural University, for his helpful advice in the statistical analysis.

要　旨：ハナキンポウゲの害虫キツネノボタンハモグリバエの個体群動態を研究するために，先ず卵，幼虫の密度および寄主植物の葉面積の推定法を検討した。

1）卵および幼虫各令期における，葉を抽出単位としたときの空間分布はいづれも集中的であることがわかった。よって資料の分散分析を行なうには予めもとの値xを

$\sinh^{-1}\sqrt{\frac{\gamma-1}{\alpha+1}x}$ に変換すべきである。

2）葉当り卵および幼虫数は枝タイプの間で有意差が認められるが，株の間には認められなかった。他方，葉当り葉面積は枝 タイプ および株の間に 有意差 が認められた。

3）株当り葉数の変動を知るために，便宜上株当りの枝数と枝当り葉数の変動を別々に検討した結果，両者とも株間，枝タイプ間に大した差はなく，したがって株当り葉数はこれらの要因によってあまり影響を受けないようである。

4）株当り葉数と葉当り虫数または葉当り葉面積の間には相関は認められない。すなわちそれぞれ２つの量は互に独立変数として扱うことができる。他方，葉当り虫数と葉当り葉面積の間にはかなり高い相関が認められた。

5）以上から株当り虫数の有効な推定値を得るには枝タイプによって，また株当り葉面積の推定値をより効率よく求めるには，技術上のはん雑を避けるために株間変動を無視して枝タイプによって母集団を２つに層別するのが望ましい。

6）以上の分析に基づいて株当りの虫数，株当りの葉面積および単位葉面積当りの虫数の推定法を示した。

References

1) Cochran, W. G. (1953) : Sampling techniques, New York : 330.

2) Ives, W. G. H. (1955) : Canad. J. Zool. **33** : 370-388.

3) Iwao, S. (1968) : Res. Popul. Fcol. **10** : 1-20.

4) Iwao, S. and E. Kuno (1968) : Ibid. **10** : 210-214.

5) Lloyd, Monte (1967) : J. Anim. Ecol. **36** : 1-30.

6) Morris, R. F. (1955) : Canad. J. Zool. **33** : 225-294.

7) Sugimoto, (1967) : Jap. J. appl. Zool. Ent. **11** : 114-118(In Japanese with English summary).

8) Yokoo, T. (1959) : 'Dojo-senchu, seitai to bojo' (Ecology and control of soil nematodes), Tokyo : 541.