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Population Estimation Techniques Studies on the Black Bean Aphid, *Aphis fabae* Scop.

Badania techniczne nad metodą oszacowania wielkości populacji gatunku *Aphis fabae* Scop.

Технические исследования над методом оценки количественного состава популяции *Aphis fabae* Scop.

The recent importance attained by the black bean aphid, *Aphis fabae* Scopoli, throughout the temperate regions of the world, has brought about the need for an accurate and practical sampling method for estimating its field populations, in order to assess its true value as a pest. The usual methods prevailing at the moment are sweepnet sampling or making random leaf counts, which, though they are practical from the point of view of determining the presence of the pest in the area, fail to give a true picture of the damage being done. Other methods, like gravimetric quantitative counts and fork sampling have been tried and recommended by various workers, like Fox and Cook (6), Gray and Schub (7), Berliński (2) and Henderson (8), but they are too laborious and time-consuming. Furthermore, the damage forecast, based on these methods, is not generally reliable, because these sampling procedures make no provision for the inclusion of variables, like the aphids' preference for certain types of leaves, the reproductive potential of the aphid both with regard to the kind of leaves and the age of the plant, the reproductive rate of the aphid, the temperature and the humidity during the sampling periods, and the presence of active predators and parasites. In view of these facts, studies were undertaken to assess statistically the value of a few different methods, prevailing at the moment, in order to find the most accurate and practical sampling method for the use of the field worker.

The main objectives of these studies were: a) to test some five different sampling techniques — Plant counts, Sticky-plant counts, Inverse binomial sampling, Leaf counts, and Fork sampling — in order to determine their accuracy and practicability for use in the estimation of the aphis population in the field,

b) to study the relative efficiency of these techniques for sampling the aphid population on the various parts of the plant, and

c) to determine the minimum number of samples and replications required for population estimation with the least degree of error.

These studies were carried out in the bean fields, infested with *Aphis fabae,* of local farms in the vales of Surrey. No special effort was made to select fields with a very high aphis population, but a random selection method was employed, making every effort to include variables like plant height, plant density, and field size. Special attention was paid to habitats of the aphid having a direct bearing on the objectives of these studies, for instance the preference of the black bean aphis for the upper portions of the plants, especially when infestations are low to moderate.

MATERIAL AND METHODS

All sampling techniques were set up on a statistical basis to include randomisation, replication, and unbiassed observations. After Cochran (4), replicates were referred to as sequences, since every replicate of each sample was taken in a uniform manner along a straight line across every field. The samples were selected at random by walking diagonally across the field and taking each sample about 20 feet apart. Each technique was evaluated on the basis of one hundred observations, comprising ten sub-samples, each replicated ten times, except for fork sampling which was evaluated from a smaller number of observations. A description of each sampling procedure is given below.

1. Inverse binomial counts

Since according to Kennedy, Ibbotson and Booth (9), Müller (10) and Tambs-Lyche and Kennedy (11) young growing and senescing leaves of *Vicia faba* L. are more susceptible to *Aphis fabae* Scop. than the mature green leaves, and, also, as the majority of these leaves (i.e. young growing and senescing ones) are present on the upper and the middle third of the plant, it follows that, in the case of low to moderate infestations, nearly the whole of the aphis population will be concentrated on these two upper portions of the plants. Therefore, for each sub-sample, the plant was sub-divided into three parts —

upper, middle and lower third — depending upon the three above mentioned types of leaves, and the aphis present on the upper and the middle portions of the plants were counted. The easiest way of counting the aphis is to cut off the plant from the base carefully, pinch off the leaves classified as mature, and to take the plant, packed in an ice cream carton, to the laboratory, where counts can be made after anaesthetising the aphids.

The counts thus made were expressed in terms of population per leaf. The record kept regarding the number of leaves per plant served to calculate the aphis population which would have been present had all of its leaves been in a proper condition to support the aphis population.

It is necessary to mention here, that, though it appears that the number of aphids arrived at by this procedure would be far greater than the actual number of aphids present on the plant, it was not so, since no significant difference was found in the actual population per plant and that calculated by this procedure.

The total number of aphids calculated per one hundred plants was utilised for analysis.

2. Plant counts

One hundred plants per field were selected at random, carefully cut at the base, and, placed in an ice cream carton, were taken to the laboratory. These plants were then sprayed with methyl isobutyl ketone, which caused the anaesthetised aphids to drop down into the receptacle, where they were collected and counted.

3. Leaf counts

Samples were obtained by selecting at random ten leaves from the upper, middle and lower portions of each plant, and counting the aphids present on them. A total number of one hundred observations per field was made in this way.

4. Sticky-plant counts

A sample for aphid counting consisted of two neighbouring plants, sprayed with a weak solution of gum arabic and examined in situ by turning over the leaves. If the aphis population was too high, two half plants were examined, while in case of very heavy infestations, observations were restricted to one-fifth of the number of leaves on each plant. It should be understood here that the phyllotaxy of the bean

plant is such that a one-fifth sample of leaves of successive ages is easily selected by following vertically up the stem from the oldest to the youngest leaves. The leaf number per plant was also recorded so that aphis counts could be reduced to a leaf basis, if required, but since the plant was taken as a unit it seemed better to keep the aphid counts on a plant basis. The counts were expressed as aphids per one hundred plants to avoid fractions where there were fewer aphids than plants.

5. Fork sampling

The equipment and technique for this procedure, first described by Henderson (8), were employed in these studies, after making some small adjustments. Details of the apparatus are given below:

The fork (Fig. 1 A) consisted essentially of four rectangular steel tines, $6^1/_2 \times {}^1/_2$ inches, and of a sufficient depth to hold single strength window glass slides, six inches long and half an inch wide (Fig. 1 B). These tines were attached to a steel plate, provided with a handle that extended from the base at an angle of 15 degrees. The triangular front points of the tines were turned slightly upwards, while bottoms of the tines were levelled, to prevent them from digging into the ground when the fork was pushed along the soil surface underneath the plants.

For convenience four slides were placed in an aluminium clip (Fig. 1 C) which had its ends turned back over the slides so that the glass surface would not touch when several clipped glass slides were stacked. Two upturned stops on the back margins of the clips prevented the slides from falling out.

Several sticky materials, like „Tanglefoot" tree banding material, supplied by Messrs. Murrey and Bright Ltd. Leeds, and „Stopmoth" tree banding grease, supplied by Messers Joseph Bentley Ltd. Bradford, were tried for coating the slides to trap the aphids falling on them, but unfortunately none of the materials proved satisfactory. The only material which did so was a hot mixture of mineral oil and vaseline (3 : 1), and this was used to coat the slides while they were held in the clip.

Samples were obtained by placing the fork under the plant, selected at random, and shaking the plant so as to allow the aphids to fall down on the glass slides, coated with mineral oil and vaseline mixture and held in the sockets of the tines. After the sample had been taken, the slides were taken out of the tines and labelled as to the sampling site, date and other necessary data. These exposed slides were then placed in clips and stacked in a temporary storing box (Fig. 1 D) provided for the purpose and taken to the laboratory where the aphids were counted.

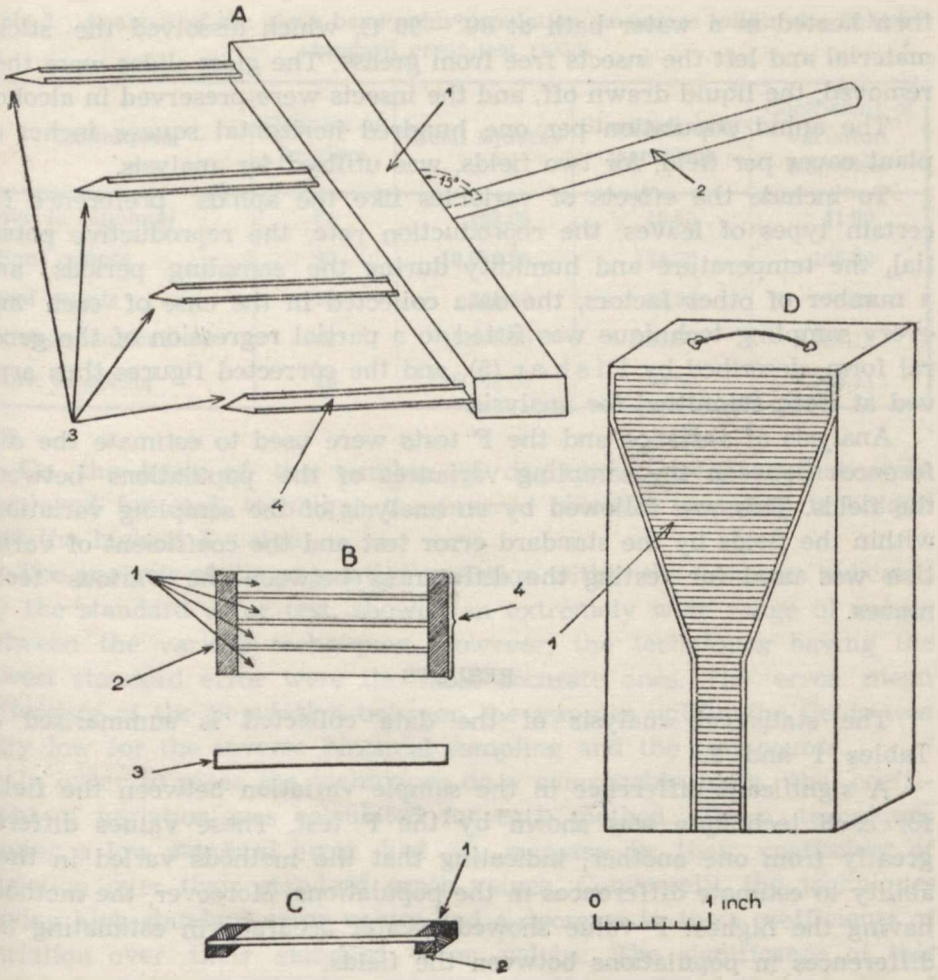

Figure 1. The fork sampling apparatus
A. 1 — base plate, 2 — handle, 3 — steel tines, 4 — tine socket for holding the glass slide;
B. 1 — glass slides. 2 — clip, 3 — glass slide, 4 — clipped slides;
C. 1 — upper side of the clip, 2 — lower side of the clip (side view of the aluminium clips while a slide is being held in them).
D. temporary storing box 1 — clipped slides.

If the time did not permit the counting of the aphids immediately after the exposed slides had been brought to the laboratory, then to save the aphids from damage, they were preserved for later counting by the following procedure.

The slides constituting one sample were placed in a beaker, and a hot mixture of equal parts of ethyl acetate and glacial acetic acid was added to cover the embedded insects. The contents of the beaker were

then heated in a water bath at 80°—90°C, which dissolved the sticky material and left the insects free from grease. The glass slides were then removed, the liquid drawn off, and the insects were preserved in alcohol.

The aphid population per one hundred horizontal square inches of plant cover per field, for two fields, was utilised for analysis.

To include the effects of variables like the aphids' preference for certain types of leaves, the reproduction rate, the reproductive potential, the temperature and humidity during the sampling periods, and a number of other factors, the data collected in the case of each and every sampling technique was fitted to a partial regression of the general form, described by Fisher (5), and the corrected figures thus arrived at were submitted for analysis.

Analysis of variance and the F tests were used to estimate the differences between the sampling variances of the populations between the fields. This was followed by an analysis of the sampling variations within the fields by the standard error test and the coefficient of variation was used for testing the differences between the various techniques.

RESULTS

The statistical analysis of the data collected is summarised in Tables 1 and 2.

A significant difference in the sample variation between the fields for each technique was shown by the F test. These values differed greatly from one another, indicating that the methods varied in their ability to estimate differences in the populations. Moreover, the methods having the highest F value showed greater accuracy in estimating the differences in populations between the fields.

Table 1. Analysis of the data for five sampling techniques on the black bean aphid, *Aphis fabae*, (1958), source of variation = between the fields.

Technique	Degrees of freedom	Mean	Mean square	„F" value
Inverse binomial counts	8	26.	1,583.98	13,3 a
Plant counts	9	127.7	63,077.33	3.50 b
Leaf counts	9	25.5	2,420.11	6.09 a
Sticky-plant counts	6	170.0	195,250.90	3.93 b
Fork sampling	1	128.0	529,238.00	29.23 a

a significant at the one percent level.
b significant at the five percent level.

Table 2. Analysis of the black bean aphis population variations within the field by standard error test (1958)

Techniques	Degrees of freedom	Mean squares	Standard error (samples)	Coefficient of variation (samples)
Inverse binomial	81	109.05	10.90	41.90
Plant counts	90	18,016.05	134.20	105.30
Leaf counts	90	398.60	19.92	78.10
Sticky-plant counts	63	49,673.60	222.70	131.10
Fork sampling	19	18,102.00	134 58	105.11

On the basis of the number of degrees of freedom that were employed for each technique the inverse binomial sampling technique had the highest F value.

The analysis of the population variation within the fields, as indicated by the standard error test, showed an extremely wide range of values between the various techniques. However, the techniques having the lowest standard error were the most accurate ones. The error mean difference of the population between the samples within the fields was very low for the inverse binomial sampling and the leaf counts.

In order to place the techniques on a comparable basis, the coefficient of variation was calculated for each method. Those techniques having a low standard error had an increase in their coefficient of variation over their standard error values. Conversely, the techniques having high standard error values had a decrease in their coefficients of variation over their standard error values. The significance of this test indicated a considerable reduction in the range of the values and thus placed these techniques on a basis for more accurate comparison. The lowest of these coefficients of variation was indicated by the inverse binomial counts.

DISCUSSION

On the basis of these different analytical tests, the inverse binomial sampling technique appeared to be the most accurate technique, although this method could not be unquestionably accepted because of a few factors involved in its practical application in the field. However, since these defects are due not to the technique itself but to the individual using the method, they could be overcome by experience.

Other important qualitative factors not measured by the statistical analysis were also given due consideration. Thus, observations on the

leaf count showed that many aphids were knocked off the leaves during the experimental procedure. A serious disadvantage was that more time was required to locate the leaves with a measurable amount of infestation, the neglect of this factor sometimes presented an altogether wrong picture.

The sticky-plant method, which was the most inaccurate technique, had several disadvantages. Firstly, the application of the gum spray knocked down a considerable number of aphids from the plant. Secondly, difference in the height and density of bean plants in many fields offset any comparison of the aphis population between and within the fields. For instance, many aphids were observed in the sparse stand while in heavy stands of plants few aphids were observed, even though the population of the aphid was the same per plant in both types of fields. This difference of growth did not affect the accuracy of the plant counts or inverse binomial sampling.

The fork sampling method was quite accurate but was time-consuming. To obtain an accurate estimate of the population, the height and density of the plants and the size of the field had to be determined. Many aphids held on to the stem while plants were being shaken, the number varying according to the individual using the method. Moreover, it was quite difficult to estimate the population accurately because of the differences in the vertical distributions of the aphids on the plants.

On the basis of these studies, it is concluded that, other conditions remaining the same, accurate aphid counts can be made by using either the inverse binomial sampling or the plant count method.

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STRESZCZENIE

W ciągu roku 1958 prowadzone były badania nad statystyczną wartością metod jakościowego szacunku populacji mszyc, *Aphis fabae* Scop., które doprowadziły do stwierdzenia, jakie z nich są dokładniejsze i praktycznie przydatne w odniesieniu do szacowania populacji mszyc.

Następujące metody były badane porównawczo: obliczanie mszyc na świeżych roślinach, obliczanie mszyc na gumowanych roślinach, przeciwieństwo binomiczne, obliczanie mszyc na ilościach, oraz „próba widełków".

Przeprowadzone badania dowiodły, że przy zachowaniu tych samych warunków populacje mszyc są mniej więcej równomiernie rozmieszczone na całej górnej powierzchni liści. Technika badań przeciwieństwa binomicznego, pomimo jej cech ujemnych w porównaniu z innymi metodami, była o wiele dokładniejsza.

РЕЗЮМЕ

В течение 1958 года велись исследования над статистической ценностью методов количественной оценки популяции тлей, *Aphis fabae* Scop. Результатом этих исследований было установление, которые из них более точны и практически более пригодны для количественной оценки популяции тлей.

Для последующего сравнения подвергались исследованию следующие методы: определяние числа тлей на свежих растениях, установление числа тлей на растениях намазанных камедью, биномильная противоположность, подсчитывание тлей на листьях, а также „fork sampling".

Произведенные исследования доказали, что при соблюдении одинаковых условий, популяции тлей более менее равномерно размещены на целой верхней поверхности листьев. Техника исследований биномильной противоположности, несмотря на ее некоторые отрицательные черты в сравнении с другими методами, оказалась наиболее точной.

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