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A Binomial Sampling Plans for *Aphis gossypii* (Hemiptera: Aphididae) in Greenhouse Cultivation of Cucumbers

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Abstract. Infestations of *Aphis gossypii* per leaf in greenhouse cultivation of cucumbers were investigated to develop binomial sampling plans. An empirical P_T-m model, $\ln(m) = \alpha + \beta\ln[-\ln(1-P_T)]$, was used to evaluate relationship between the proportion of infested leaves with $\leq T$ aphids per leaf (P_T) and mean aphid density (m). Tally thresholds (T) were set to 1, 3, 5, 7, and 9 aphids per leaf to find appropriate T in greenhouse cultivation of cucumbers. Increasing sample size had little effect on the precision of the binomial sampling plan. However, the precision increased with tally threshold. The binomial model with $T = 5$ provided appropriate predictions of the mean densities of *A. gossypii* in the greenhouse cultivation of cucumbers. Using a binomial model with $T = 5$ (sample size = 200), a wide range of densities (1.2 - 222.8 aphids per leaf) could be estimated with precision levels of 0.346 - 0.380 for P_T values between 0.15 and 0.96. Binomial models were validated at $T = 5$ and 7 using 12 independent data sets. Both binomial models were robust and adequately described aphid densities; most of the independent sampling data fell within 95% confidence intervals around the prediction model.

Additional key words**:** cotton aphid, tally threshold

Introduction

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), causes serious damage to commercial greenhouse cultivation of cucumbers if proper control methods are not applied in a timely manner. *Cucumis sativus* L., is an important horticultural crop in Korea. However, overuse of monotype insecticides can induce rapid development of insecticide resistance and thus led to control failure (Silver et al., 1995). Thus, an environment-friendly method to control *A. gossypii* is required, such as biological control agent or an integrated pest management scheme (IPM).

In IPM programs, the sampling method is used to determine the density of the pest species is of critical importance (Bligaard, 2001). Sampling plans determine the size, number, and spatial arrangement of the sampling units of target pests in specific cultivation system. Demand for rapid and reliable sampling methods for insect pests has increased with the increased implementation of pest management programs in recent years (Shepard, 1980). To design a sampling program, the allocation of sampling resources and costs are considered the most important steps. In terms of management decisions, information about a population should be collected in the shortest amount of time with minimum cost, yet still be highly reliable.

In general, complete enumerative sampling methods are complicated and time-consuming because large sample size and numbers are required to precisely estimate the insect densities, especially due to the large variance introduced by aggregated distributions of insects. Thus, several studies have attempted to develop efficient and reliable sampling plans to estimate the abundance or damage levels of various insect pests (Baek et al., 2009; Cho et al., 2001; Jones, 1994; Kapatos et al., 1996; Kim et al., 2001; Kuno, 1991; Nyrop et al., 1989; Park and Tollefson, 2006).

Sampling plans for decision-making in IPM strategies must minimize both the error in inaccurately estimating densities and the cost of gathering the information (Pedigo, 1994).

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Relatively little effort has been made to devise viable sampling strategies for cotton aphids in greenhouse cultivation of cucumbers. Our goal in the current study was therefore to evaluate the utility of binomial sampling methods for *A. gossypii* densities in commercial greenhouse cultivations of cucumbers.

Materials and Methods

Sampling

Densities of *A. gossypii* in commercial greenhouse cultivation of cucumbers were surveyed which was located in Gyeonggi-do, Korea (latitude: 37.072447°, longitude: 127.032574°) in 2003 and 2005. In the greenhouse (3,300 m^2; 60 × 55 m), about 6,000 cucumber seedlings were heavily planted at regular intervals (0.25 - 0.3 m) in mid-February, and the greenhouse was artificially heated by an agricultural heating systems until early April.

All the plants were positioned with wire directly over the rows of plants at a height of 1.8 - 2.0 m. Each plant was trained vertically along the support plastic twine and then tied with plastic snap-on clips. As the plants grew up and reached to the height of top supporting wire, the plastic clips were released, and each plant was let down by about 0.3 m, and then the plastic clips were s refastened. When lower parts of the plants and the old foliage touched the ground, the lowest foliage was periodically removed to promote the flowering and fruit production. During the periods of investigations, all the treatments were conducted based on the grower's preferences concerning pest and disease control.

One hundred twelve points were marked by about 5 m intervals in the greenhouse, and the densities of *A. gossypii* on the third and sixth leaves from the bottom of each plant were investigated at intervals of 7 - 10 days. Sampling points (cucumber plants) were randomly selected within 1-m range from the initial marking point.

Development of a Binomial Sampling Plan

The empirical relationships between the mean densities (m) of *A. gossypii* on leaves and the proportion of infested leaves (P_T) with at least T aphids per leaf were determined by linear regressing $\ln(m)$ on $\ln[-\ln(1-P_T)]$ (Kono and Sugino, 1958), as follows:

$$\ln(m) = \alpha + \beta \ln[-\ln(1-P_T)] \qquad \text{Eq. 1}$$

Where α and β are estimated by linear regression (PROC GLM, SAS Institute, 2004). The model parameters were estimated for tally thresholds (T) of 1, 3, 5, 7, and 9 aphids per leaf, residuals were examined, and lack-of-fit statistical tests were performed.

To evaluate and compare the binomial models based on different tally thresholds, the variance of the mean (var(m)) predicted from the proportion of sample units infested were compared. The calculation of a valid variance for a mean prediction is necessary to evaluate the precision of binomial sampling programs (Binns and Bostanian, 1990). The var(m) predicted from the proportion of the infested sample units (Schaalje et al., 1991) was estimated as follows:

$$\begin{aligned}
&\mathrm{var}(m) = m^2[c1 + c2 + (c4 - c3)] \\
&c1 = (\beta^2 P_T)/n(1-P_T)\ln(1-P_T)^2 \\
&c2 = MSE/N + \{\ln[-\ln(1-P_T)] - P_m\}^2 s_\beta^2 \qquad \text{Eq. 2} \\
&c3 = \exp[\ln a + (b-2)[\alpha + \beta \ln(-\ln(1-P_T))]]/n \\
&c4 = MSE
\end{aligned}$$

Where MSE is the mean square error from Eq. 1, N is the number of data points in the regression used to estimate α and β from Eq. 1, P_m is the average value of ln[- ln(1 - P_T) used in the regression, S_β^2 is the sample estimate of variance; β, n is the number of samples from a population, and a and b are from $\ln s^2 = \ln a + b \ln m$ and describe the relationship between the variance (s^2) and mean (m) (Taylor, 1961). The homogeneity of Taylor's regression slopes and the equality of the intercepts between greenhouses were tested using an analysis of covariance (ANCOVA) (Sokal and Rohlf, 1981). If no significant differences were detected, data were pooled for a common Taylor's power law regression (SAS Institute, 2004).

Defining the precision (d) as the standard error to the mean ratio, $d = (s^2/n)^{0.5}/m$, and substituting Eq. 2 for s^2/n gives (Nachman, 1984):

$$d = \sqrt{[(c1 + c2 + (c4 - c3))]} \qquad \text{Eq. 3}$$

Validation and Classification of the Sampling Plan

The precisions of the binomial sampling programs were evaluated and compared among different models as a function of the mean density for sample size 50, 100, 150, and 200 leaves.

The performance of the binomial sampling plan was evaluated using the 12 independent data sets that had not been used to develop the binomial sampling plan. Independent data sets were obtained at another commercial greenhouse in 2006 and for greenhouse cucumbers (300 m^2) grown by the National Institute of Horticultural & Herbal Science (NIHHS) in 2007. We evaluated whether the 12 data sets,

Table 1. Parameters of an empirical binomial model $\ln(m) = \alpha + \beta\ln[-\ln(1 - P_T)$ relating the mean number of aphids per leaf to the proportion of leaves infested at least *T* aphids.

T	α	β	N	r^2	P_m	$S^2\beta$	*MSE*	P_T = 0.95[z]	P_T = 0.05
1	3.303	1.419	39	0.66	-1.927	0.0276	0.1271	129.0	0.4
3	3.822	1.328	39	0.77	-2.450	0.0146	0.1060	196.2	0.9
5	4.003	1.279	38	0.79	-2.619	0.0121	0.0966	222.8	1.2
7	4.099	1.219	37	0.80	-2.765	0.0108	0.0922	229.6	1.6
9	4.150	1.146	37	0.82	-2.986	0.0080	0.0916	223.0	2.1

[z] Mean density with 95% and 0.5% of the sample units infested.

when analyzed using the binomial sampling plan, fell within 95% confidence limits for the prediction model (Eq. 4) at *T*. The confidence limits of a prediction mean (m) on a logarithmic scale were computed using Eq. 4 (Jones, 1994).

$$\ln(m) \pm z_{\alpha/2}\sqrt{Var\,(\ln(m))} \qquad \text{Eq. 4}$$

Decision lines for sequential classification binomial sampling plans (Eq. 5) were calculated using the equations of Nyrop et al. (1989):

$$UL = nP_T + z_{\alpha/2}n\{[P_T(1-P_T)]/n\}^{0.5}$$
$$LL = nP_T - z_{\alpha/2}n\{[P_T(1-P_T)]/n\}^{0.5} \qquad \text{Eq. 5}$$

Where *UL* is upper stop line (stop sampling and carry out management action) and *LL* is lower stop line (stop sampling and do not carry out management action). If the result lies between *UL* and *LL*, sampling must proceed. In these equations, n is the sample size, and P_T is the proportion of infested leaves with at least *T* aphids per leaf based on each action threshold. A value of 1.96 was used for $z_{\alpha/2}$ at a confidence level of 95% (parameter of normal distribution).

Results

Of the total 40 separate estimates of sampling data (mean densities; 0.07 - 80.39 aphids/leaf), 37 - 39 estimates of P_T were useful for the development of a binomial sampling plan after eliminations of the values of 0 and 1. The empirical $P_T - m$ model (Eq. 1) fitted the data sets relatively well, and as *T* increased, so did the r^2 value, ranging from 0.66 to 0.82 (Table 1 and Fig. 1). Examination of the residual errors from the regression indicated that the model fits improved as the *T* increased (Table 1). The parameters of the Taylor's power law were estimated from the pooled data because the slopes (*b*) and intercepts (ln(*a*)) of each regression line did not differ significantly between greenhouses and locations (ANCOVA, $p > 0.05$). The common regression equation for Taylor's power law was $\ln s^2 = 3.223 + 1.758\ln m$, with r^2 = 0.92.

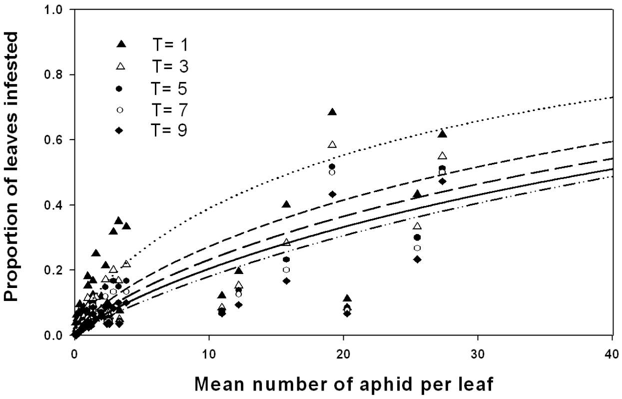

Fig. 1. Relationship between the proportion of leaves infested with at least *T* aphids and the mean number of aphids per leaf with *T* = 1, 3, 5, 7, and 9.

To find upper and lower limit of the mean density for each binomial model, the proportions of infested leaves were presented as a function of the mean density at the different tally thresholds. The mean densities estimated at P_T = 0.95 were 129.0, 196.2, 222.8, 229.6, and 223.0, aphids per leaf at *T* = 1, 3, 5, 7, and 9, respectively. The mean densities estimated at P_T = 0.05 were 0.4, 0.9, 1.2, 1.6, and 2.1 aphids per leaf at *T* = 1, 3, 5, 7, and 9, respectively (Table 1 and Fig. 1). Therefore, all the tally thresholds permitted estimation of mean upper limit values equivalent to the maximum densities observed during the sampling dates.

The effects of the different tally thresholds on the estimation of the mean density were examined by determining the sampling precision (*d*) as a function of the proportion of leaves infested and the sample size (Fig. 2), using Eq. 3 and the parameters in Table 1. We found that the tally threshold had a drastic effect on the sampling precision, where sampling size had a little effect on the sampling precision (Fig. 2). This appears to be due to the relatively low impact of the sample size on the variance term (Jones, 1994). However, the precision level never exceeded ≈0.30. Precision levels of 0.346 - 0.380 were obtained for P_T values

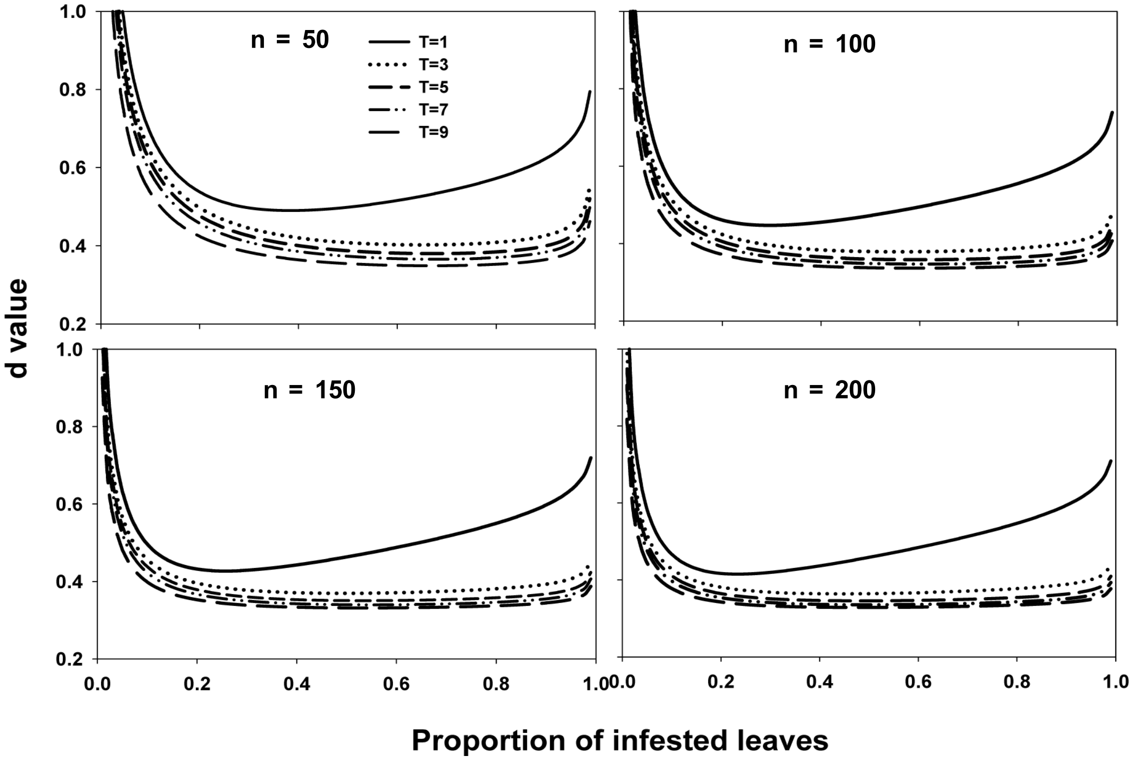

Fig. 2. The precision (SE/mean) achieved when estimating the mean density from the proportion of sample units infested with at least *T* aphids for sample size of 50, 100, 150, and 200 leaves.

between 0.15 and 0.96 at the tally threshold (*T*) of 5 with a sample size of 200. Similar results were observed for *T* = 7 and 9. We attributed these results to the stabilities of the *MSE* (Table 1) and to the fact that the *MSE* is the largest variance component (Binns et al., 2000).

Stop the limits of decision-making for management of *A. gossypii* were calculated using the 95% confidence intervals based on action thresholds of 20, 40, and 80 aphids per leaf (tally threshold ≥ 5 aphids per leaf) (Table 2). The proportion of infested leaves (P_T) with at least 5 aphids per leaf based on action thresholds (m_T) 20, 40, and 80 were 0.266, 0.378, and 0.517, respectively. For accurate decision-making, the probabilities must show a normal distribution, namely $np > 5$ and $n(1-p) > 5$. Therefore, the sample size should be larger than 10 leaves (Table 2).

Management action could be decided easily using the stop limits estimated by counting the number of samples with a tally threshold ≥ 5 aphids per leaf. Thus, if the cumulative number of leaves with a tally threshold greater than the *UL* or less than *LL* is reached, sampling can be stopped. If the cumulative number of leaves still lies between the *LL* and *UL*, then sampling should be continued until the maximum number of samples is reached.

The relationship between the mean density and the proportion of infested sample units were evaluated at *T* = 5 and 7. Both binomial models (*T* = 5 and 7) were robust

Table 2. Stop limits of decision-making for management *A. gossypii* by using 95% confidence intervals based on action thresholds of 20, 40, and 80 aphids per leaf (tally threshold; ≥ 5 aphids per leaf).

No. of leaves sampled	Action thresholds(aphids per leaf; m_T)					
	m_T = 20		m_T = 40		m_T = 80	
	LL[z]	UL	LL	UL	LL	UL
10	-	-	-	-	-	-
20	2	10	4	12	6	15
30	4	13	7	17	11	21
40	6	17	10	22	15	27
50	8	20	13	26	19	33
60	10	23	16	31	24	39
70	12	26	19	35	28	45
80	14	30	22	39	33	51
90	16	33	25	43	38	56
100	18	36	29	48	42	62
110	21	39	32	52	47	68
120	23	42	35	56	52	73
130	25	45	39	60	57	79
140	27	48	42	65	61	84
150	30	51	45	69	66	90
160	32	54	49	73	71	96
170	34	57	52	77	76	101
180	37	60	56	81	80	107
190	39	63	59	85	85	112
200	41	66	63	89	90	118
	Stop sampling					

[z] Lower limit and upper limit, respectively.

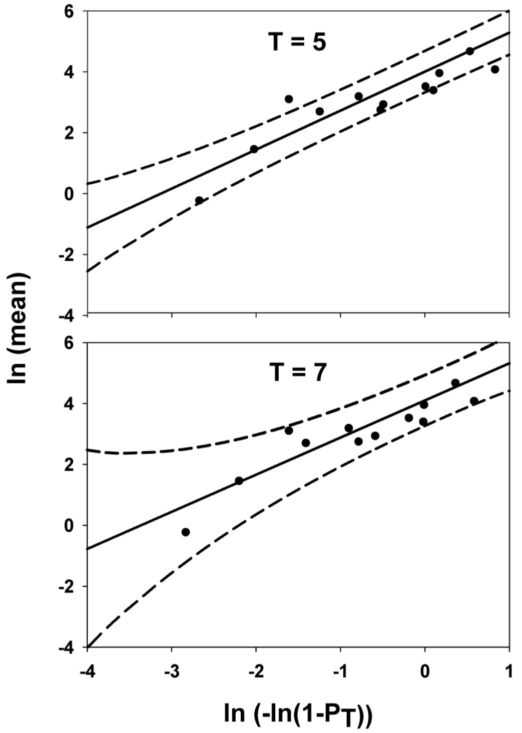

Fig. 3. The fits of the Kono and Sugino models for independence data sets using two different tally thresholds (T = 5 and 7). The dotted lines represent 95% confidence intervals around the predicted equation (solid line).

and adequately described the data, most of the independent observation sampling data fell within the 95% confidence intervals around the prediction model (Fig. 3).

Discussion

Binomial sampling (presence-absence sampling) has been described as a short-cut for estimating population densities (Anscombe, 1948; Nachman, 1984). This type of sampling scheme is usually founded on defining a relationship between the proportion of sampling units occupied by one or more individuals and the mean number of individuals per plant (Binns and Nyrop, 1992). When performing binomial samplings, it is necessary to investigate the presence or absence of pests on more sampling units than tally threshold (T) and to calculate two variables (P_T and m) to perform linear regressions analysis. The binomial sampling method contains more variables than did the complete counts methods (Eq. 1). Furthermore, considering the trade-off between binomial and enumerative samplings, the precision of the binomial sampling plan is generally lower than that of complete enumerative sampling. Estimates based on binomial samplings are also generally more variable than estimates based on enumerative samplings and thus may be less precise (Binns and Nyrop, 1992). Therefore, more samples are usually necessary to achieve the desired precision.

The advantages of binomial sampling are its speed and simplicity, which in many cases outweigh the larger number of samples required. Binomial sampling has been successfully used in a large number of pest management systems for a variety of pests (Binns and Bostanian, 1990). Another merit of the binomial sampling method is that it is less likely to be affected by highly skewed abnormal data relative to complete enumerative methods (Cho and Park, 1997). In complete enumerative sampling methods, the presence abnormally high data could have a large effect on the estimated variations and mean densities of the pest species of interest. In the binomial sampling method, pest densities are estimated on the ratio of infested sampling unit greater than a tally threshold, so abnormally high densities would have a negligible effect on the results. Selection of a proper tally threshold in binomial sampling can reduce the variability and bias of the estimated mean, and use of sequential classification can yield acceptable error rates and average sample sizes (Cho et al., 2000; Lee et al., 2005; Nyrop and Binns, 1991; Song, 2003).

A binomial sampling strategy is more practical for devising action plans for pest management because multiple samples could be investigated within a short time. Furthermore, binomial sampling plans are suitable for samplings of small insect pests that are difficult to enumerate and those that show clumped distributions. For example, Wilson and Room (1983) reported that it took only about 1 minute to sample mites on cotton leaves by binomial sampling, but it took more than 2 hours by complete enumerations. The lower precision of binomial sampling compared to that of complete enumeration can be addressed by adequate adjustment of the tally threshold (T) during the binomial sampling process.

In general, if a high tally threshold (T) is chosen, decision-making for pest management takes more time and is more costly, thereby negating the advantages of a binomial sampling methods (Binns et al., 2000). Therefore, the tally threshold (T) should be carefully chosen by considering costs and benefits as well as the level of precision required. It should be also noted that the upper limit of the mean density can be estimated before the binomial sampling model became asymptotic (Naranjo et al., 1996). In our study, the mean densities that were estimated near the asymptote (P_T = 0.95) were 222.8 and 229.6 aphids per leaf at T = 5 and 7, respectively (Table 1). These estimated density ranges were generally higher than those observed during the sampling periods in commercial greenhouse cucumbers. Increasing the sample size from 50 to 200 had little effect on the sampling precision, regardless of the tally threshold (Fig. 2). This finding is consistent with those of previous studies (Cho et al., 1998; Lee et al., 2005; Salguero Navas et al., 1994;). This is likely because sample size has little effect on the estimation of the variance term (Jones, 1994) and the *MSE* is the largest variance component (Binns et al., 2000).

In our study, regardless the tally thresholds (1 - 9) or sample sizes (50 - 200), we did not obtain the level of precision ($d \leq 0.30$) suggested by Southwood (1978). However, the binomial models with a tally threshold of 5 and 7 were robust and adequately described most of the independent observations; the data fell within the 95% of confidence intervals of the prediction (Fig. 3). Based on the above analysis, binomial models at $T = 5$ or 7 appear to be the best for estimation the mean densities of *A. gossypii* on greenhouse cucumbers. In general, the variance in estimating the mean density declined with increasing T to a certain point (Binns and Bostanian, 1990; Cho et al., 2000; Lee et al., 2005; Naranjo and Hutchison, 1997). Selection of an appropriate tally threshold is the most important consideration for economically estimating with precision the densities of aphid within a wide range of densities when using a binomial sampling method (Cho et al., 2000).

In our study, differences in the time required to count pests on the sampling unit were not investigated in relation to the different tally thresholds. However, Nyrop and Binns (1991) found that the increase in time spent counting for different tally threshold may not be as significant. For example, time spent sampling European red mite at different tally thresholds only doubled as tally thresholds increased from 1 to 8. Therefore, in many cases, increasing the tally threshold of the sampling unit (additional time consuming) could yield greater benefits than complete enumerative sampling (increased accuracy for decision making). The binomial sampling plan presented here should greatly enhance the efficiency of monitoring *A. gossypii* densities in the greenhouses and facilitate rapid decision-making. Additional studies that evaluate the trade-off among precision, sampling time, and the costs of decision-making are required.

In conclusion, a binomial sampling plan can be practically applied to obtain a rapid estimate of the *A. gossypii* densities on greenhouse cucumbers with minimal costs. A more efficient sampling plan could potentially be devised if the distribution patterns of *A. gossypii* within and between plants in the greenhouses were taken into consideration in the binomial sampling plan.

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