Original article KOREAN JOURNAL OF APPLIED ENTOMOLOGY

한국응용곤충학회지
Korean J. Appl. Entomol. 55(4): 405-412 (2016)
DOI: http://dx.doi.org/10.5656/KSAE.2016.10.0.049

pISSN 1225-0171, eISSN 2287-545X

Development of a Binomial Sampling Plan for *Bemisia tabaci* in Paprika Greenhouses

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파프리카온실에서 담배가루이의 이항표본조사법 개발

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ABSTRACT: Infestation of adults and pupae of sweetpotato whitefly, *Bemisia tabaci*, on paprika (*Capsicum annuum* var. *angulosum*) grown in greenhouses in Jinju, Gyeongnam province during 2014was determined by counts of the number of target stage of *B. tabaci* per leaflet. Binomial sampling plans were developed based on the relationship between the mean density per leaflet (m) and the proportion of leaflets infested with less than T whiteflies (P_T), according to the empirical model (($\ln(m) = a + \beta(\ln(-\ln(1-P_T)))$). T was defined as the tally threshold, and set to 1, 2, 3, 4, 5 (adults) and 1, 3, 5, 7 (pupae) per leaflet in this study. Increasing the sample size, regardless of tally threshold, had little effect on the precision of the binomial sampling plan. Based on the precision of the model, T = 1 was chosen as the best tally threshold for estimating densities of *B. tabaci* adults and T = 3 was best tally threshold in *B. tabaci* pupae. Using the results obtained in the greenhouse, a simulated validation of the developed sampling plan by RVSP (Resampling Validation for Sampling Plan) demonstrated the plan's validity. Above all, the binomial model with T = 1 and T = 3 provided reliable predictions of the mean densities of *B. tabaci* adults and pupae on greenhouse paprika.

Key words: Paprika greenhouse, *Bemisia tabaci*, Binomial sampling plan, Tally threshold

초 록: 경남 진주시 대곡면에 위치한 파프리카(*Capsicum annuum* var. *angulosum*) 온실에 피해를 주는 해충인 담배가루이(*Bemisia tabaci*) 성충과 번데기의 밀도를 엽 당 해충 수로 2014년 조사하였다. 이항표본조사법은 엽 당 담배가루이 성충과 번데기의 밀도(m)와 담배가루이 성충과 번데기가 T마리보다 많이 존재하는 잎의 비율(P_T)과의 관계를 기본으로 하며, T는 경험적 이항분포모형($\ln(m) = a + \beta(\ln(-\ln(1-P_T)))$)에서의 tally threshold로서 본 연구에서는 성충의 경우 1, 2, 3, 4, 5 그리고 번데기는 1, 3, 5, 7을 사용하였다. 표본수 증감은 T와 관계없이 이항분포 모형의 정확도에 영향이 거의 없었다. 이항분포모형의 정확도는 성충의 경우 T=1 일 때, 번데기의 경우 T=3일 때 가장 높았으며, 최적의 tally threshold인 것으로 나타났다. 마지막으로 분석에 사용하지 않은 독립된 자료를 이용하여 개발된 표본조사법의 유효성을 Resampling Validation for Sampling Plan (RVSP) 프로그램으로 평가하였다. 그 결과 파프리카 온실에서 담배가루이 성충과 번데기의 밀도추정에는 T=1 (담배가루이 성충), T=3 (담배가루이 용)인 경우가 적합한 것으로 판단되었다.

검색어: 파프리카 온실, 담배가루이, 이항표본조사법, Tally threshold

The sweetpotato whitefly, *Bemisia tabaci*, is an economically important agricultural pest which attacks about 500 host plants including both vegetable and ornamental plants. This pest is known to have more than 24 biotypes, among which the B and Q biotypes cause the greatest damage to host plants (Van Lenteren and Woets, 1988; Perring et al., 1993). The occurrence of the B-biotype of *B. tabaci* in Chungbuk province, Korea was confirmed in 1998. The Q-biotype was discovered in Gyeongnam and Jeonam provinces in 2004 (Lee et al., 2005). *B .tabaci*

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Received August 29 2016; **Revised** October 27 2016
Accepted October 31 2016
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causes plant destruction by sucking nutrients from leaf surfaces and depositing sticky honeydew which leads to growth of sooty mold on leaf surfaces (Byrne, 1999). *B. tabaci* causes additional serious problems by transmitting more than 100 viral diseases such as tomato yellow leaf curl virus (TYLCV) (Muñiz and Nombela, 2001; Jones, 2003; Lee et al., 2005; Navas-Castillo et al., 2011).

In order to control *B. tabaci* in paprika greenhouses, farmers depend mainly on pesticide applications. However, pesticides which can be used on products grown for exports are limited, and the insects develop pesticide resistance over time (Kim, 2009).

Because *B. tabaci* life cycle is very short, pesticide resistance appears faster than it does with other insect pests. *B. tabaci* develops resistance against organophosphorous, cabamate, IGR, pyrathroid and neonicotinoid making pest management difficult. Because of the high likelihood of developing a pesticide resistant population and the problem of safe agricultural exports, a system of integrated pest management (IPM) is required to control insect pests (Elhag and Horn, 1983; Ahn et al., 2001; Park et al., 2014).

In order to use IPM, insect density estimation should be accurate. And density should be compared with specific injury and control levels (Song et al., 2001). Efficient methods of insect pest estimation and classification of injury level use the binomial sampling plan. Because this kind of sampling plan employs various sample units, its cost is less than a fixed-precision level sampling plan (Waters, 1955; Sterling, 1975).

The objectives of this study were to develop and evaluate binomial sampling plans for estimating the population density of *B. tabaci* in a paprika greenhouse.

Materials and Methods

The survey locations and sampling position

This study was conducted in paprika greenhouses in Jinju, Gyeongnam province. The area of the sampled greenhouse is 12,000 m^2 (Greenhouse 1 (GH 1), width × length, 120 m × 100 m). Approximately 2,160 plants were grown in rock wool media by the modified vertical codon system. The paprika plants were grown to 2.0 m~2.5 m height from the ground. Pest management included pesticide and conventional culture. Nineteen sampling positions in the greenhouse were selected. Fifteen of the 19 sampling positions were selected at spaces of 30 m (width) by 50 m (length, in case of both ends and center row). In the second and fourth selected rows, another four sampling positions were selected for improving the fit (Fig. 1-A). To obtain independent data for validation of the developed sampling plan, we selected a greenhouse that cultivates paprika in a similar area and with a similar method. The size of this paprika greenhouse is 5,000 m^2 (Greenhouse 2 (GH 2), width × length, 50 m × 100 m). Since GH 2 is smaller than GH 1, we selected similar 9 sampling positions (intervals of 20 m (width), 50 m (length)) (Fig. 1-B). Sampling was performed 22 times every week from January 2014 to June 2014.

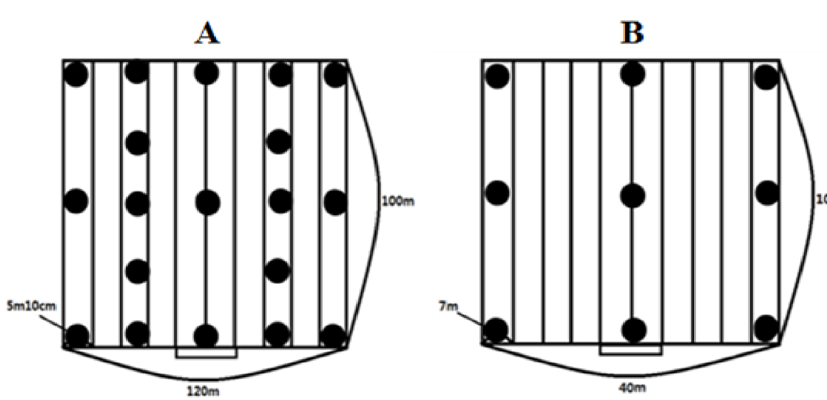

Fig. 1. The diagrams of two paprika greenhouses. 19 sampling position was chosen in Greenhouse A which sampled for development binomial sampling plan, whereas Greenhouse B where 9 sampling positions were selected for the data of validation of binomial sampling plan which developed in Greenhouse A.

Sampling

Plants in the greenhouse were divided into three positions: top (180~220 cm from the ground), middle (80~120 cm from the ground) and bottom (30~70 cm from the ground) positions. *B. tabaci* adults and pupae were observed on three paprika leaves at each position and recorded. This greenhouse used the modified vertical codon system. Because the height of the paprika plants was over 2 m when this study was begun, similar stages of *B. tabaci* always existed at similar positions on the plant. *B. tabaci* adults moved around on the top of the plants, usually laying on the sprout. Because some *B. tabaci* moved to the bottom of the plant after oviposition, most *B. tabaci* adults were distributed on the top and bottom positions. In case of *B. tabaci* pupae, as the plants grow the leaves with deposited eggs were on the bottom position. The hatched larvae had almost no movement (Kim, 2009). Thus *B. tabaci* pupae were distributed on the middle and bottom positions. Therefore, in this study *B. tabaci* adults were sampled on the top and bottom positions, and pupae were sampled on the middle and bottom positions.

Development of the Binomial Sampling Plan

The empirical model of Kono and Sugino (1958) was used to determine the relationship between mean density (*m*) and the proportion of sampling units infested with at least *T* individuals (P_T). The model is:

$$\mathrm{In}(m) = \alpha + \beta(\ln(-\ln(1-P_T))) \quad \text{Eq. (1)}$$

where α and β are parameters estimated by the linear regression (PROC GLM. SAS Institute, 1995). The model parameters were estimated for different tally thresholds (*T*) of 1, 2, 3, 4, 5 for *B. tabaci* adults and 1, 3, 5, 7 for *B. tabaci* pupae per leaf. Calculating a valid variance of a predicted mean is required to validate the precision of the binomial sampling plan (Binns and Bostanian, 1990). There are several ways to estimate this variance. In this study, the var (*m*) predicted from the proportion of sample units infested was estimated according to the method of Schaalje et al. (1991). This method was is used to evaluate and compare the precision of the binomial sampling plan according to the tally threshold:

$$\mathrm{var}(\ln m) = (\mathrm{c1} + \mathrm{c2} + (\mathrm{c4} - \mathrm{c3}))$$

$$\mathrm{c1} = (\beta^2 P_T)/n(1-P_T)\ln(1-P_T)^2$$

$$\mathrm{c2} = \frac{MSE}{N} + (\ln(-\ln(1-P_T)) - \overline{P})^2 s^2{}_\beta \quad \text{Eq. (2)}$$

$$\mathrm{c3} = \exp(\ln a + (b-2)(\alpha + \beta\ln(-\ln(1-P_T))))/n$$

$$\mathrm{c4} = MSE$$

where MSE = the mean square error from Eq. (1), N = the number of data points in the regression used to estimate α and β Eq. (1), $\overline{P}$= the average value of $\ln(-\ln(1-P_T))$ used in the regression, $s^2{}_\beta$= the sample estimate of variance of β, n = the number of samples taken from a population. The parameters *a* and *b* of Taylor's power law (Taylor, 1961) were taken from Choi and Park (2015) with a = 2.15, b = 1.30 (*B. tabaci* adults) and a = 8.72, b = 1.48 (*B. tabaci* pupae), respectively.

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

$$d = \sqrt{(\mathrm{c1} + \mathrm{c2} + (\mathrm{c4} - \mathrm{c3}))} \quad \text{Eq. (3)}$$

Validation of Sampling Plans

The accuracy of the binomial sampling plan was evaluated by using the independent data sets that had not been used in developing the binomial sampling plan. The evaluation involved determining whether the six independent data sets fell within the 95% confidence limits for the prediction model (Eq. (1)) at tally thresholds of 1 (*B. tabaci* adults) and 3 (*B. tabaci* pupae). The confidence limits of a prediction mean (*m*) on the logarithmic scale were computed using $\ln(m) \pm z_{a/2}\sqrt{Var(\ln m)}$ ($z_{\alpha/2}$ = 1.96) (Jones, 1994). We used Fixed Sample Size (FSS) and Wald's Sequential Probability Ratio Test (SPRT) of Resampling Validation for Sampling Program (RVSP). There were 500 simulations for each independent set. The action thresholds used were 2 (*B. tabaci* adults) and 10 (*B. tabaci* pupae) per leaf (Choi and Park, 2015). In FSS, we compared the actual mean with the predicted mean at *T* = 1 (*B. tabaci* adults) and *T* =3 (*B. tabaci* pupae). In SPRT, the upper bound and lower bounds used 0.45 and 0.25, respectively, and α and β errors used 0.1 in all simulations.

Table 1. Parameters of an empirical binomial model $\mathrm{In}(m) = \alpha + \beta(\ln(-\ln(1-P_T)))$ relating mean *B. tabaci* adults and pupae per leaf to the proportion of leaves infested with more than *T B. tabaci*

Stage	T	α	β	N	r^2	$\overline{P}$	s^2_{β}	*MSE*
Adults	1	0.92779	0.70321	22	0.92	-1.3898	0.0019	0.0321
	2	1.18135	0.53351	22	0.86	-2.3072	0.0021	0.0580
	3	1.38266	0.49387	22	0.87	-2.9000	0.0018	0.0574
	4	1.66876	0.51171	19	0.82	-3.0665	0.0031	0.0579
	5	2.29451	0.66616	17	0.84	-3.1894	0.0054	0.0506
Pupae	1	1.90069	0.79415	22	0.83	-1.7534	0.0060	0.0917
	3	2.15999	0.75162	21	0.81	-2.0485	0.0068	0.0557
	5	2.02137	0.56897	21	0.80	-2.4624	0.0040	0.0565
	7	2.28483	0.62900	21	0.81	-2.6463	0.0048	0.0562

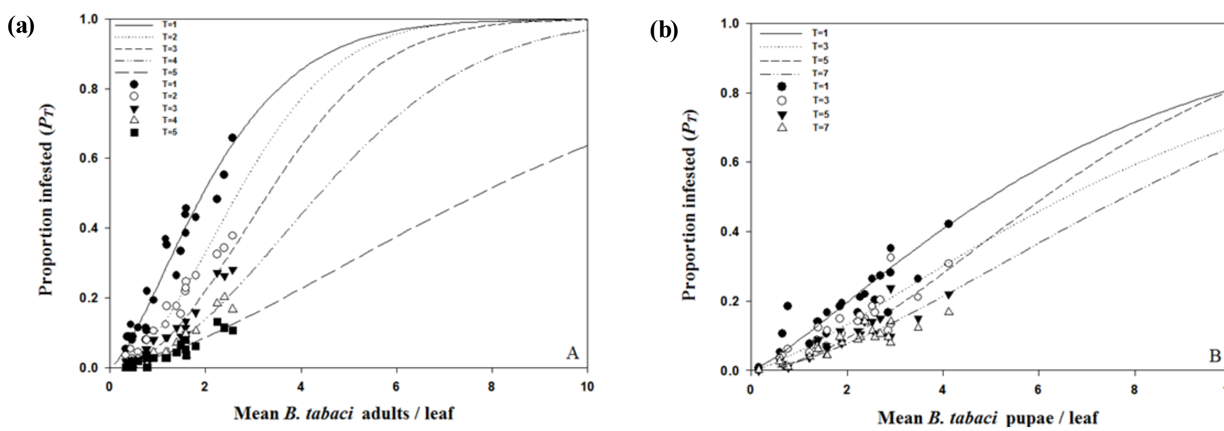

Fig. 2. Relationship between the proportion of sample unit infested and the mean number of *B. tabaci* adults per leaf with T=1, 2, 3, 4, 5 and *B. tabaci* pupae per leaf T = 1, 3, 5 and 7.

Results and discussion

Binomial sampling plan

A total of 22 consecutive week data sets, which were split between *B. tabaci* adults and pupae densities, were collected and recorded during this study. However, because of the very low densities in each greenhouse, we used 17-22 weeks data for adults and 21-22 weeks data for pupae to develop the binomial sampling plan, after elimination of the P_T value of 0 and 1, depending on the tally thresholds (Table 1). The data from the top and bottom positions (*B. tabaci* adults) and the middle and bottom positions (*B. tabaci* pupae) on the plants were combined and the infested proportions calculated. Spatial distribution patterns (Choi and Park, 2015) and infestation rates were similar to each other. Thus, the binomial sampling plan is equally applicable to the top and bottom positions on the plants for *B. tabaci* adults and to the middle and bottom positions on the plants for *B. tabaci* pupae.

The relationship between the proportion of infestation for T = 1, 2, 3, 4, 5 and the observed means for m > 2.5 of *B. tabaci* adults is shown in Fig. 2 (a). Fig. 2 (b) shows the relationship between the proportion of infestation for T = 1, 3, 5, 7 and the observed means for m > 4.1 of *B. tabaci* pupae in Fig. 2 (b). The r^2 values ranges of this empirical binomial model were 0.82-0.96 for adults and 0.80-0.83 for pupae. The highest r^2 value was observed at T = 1 (*B. tabaci* adults and pupae). Investigation of the residual error from the regression (*MSE*) shows that the model fit improves up to T = 1 (*B. tabaci* adults) and T = 3 (*B. tabaci* pupae). This suggests that the binomial model fits best at T = 1 (*B. tabaci*'s adults) and T = 3 (*B. tabaci* pupae).

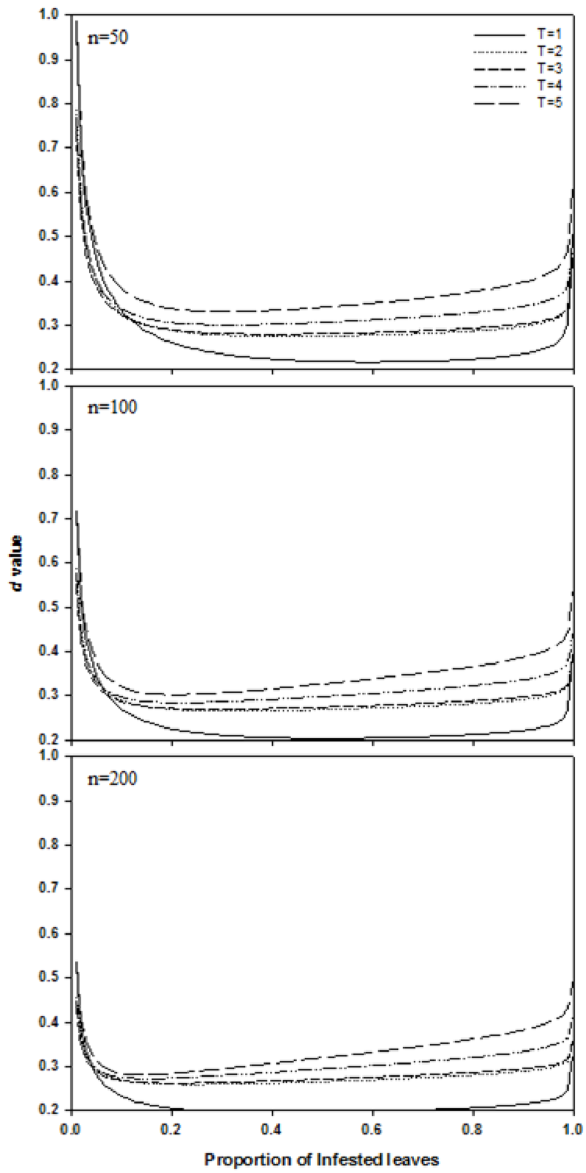

Fig. 3. The sampling precision (*d*) expressed as a function of proportion of infested at the sample size of 50, 100 and 200 (*B. tabaci* adults).

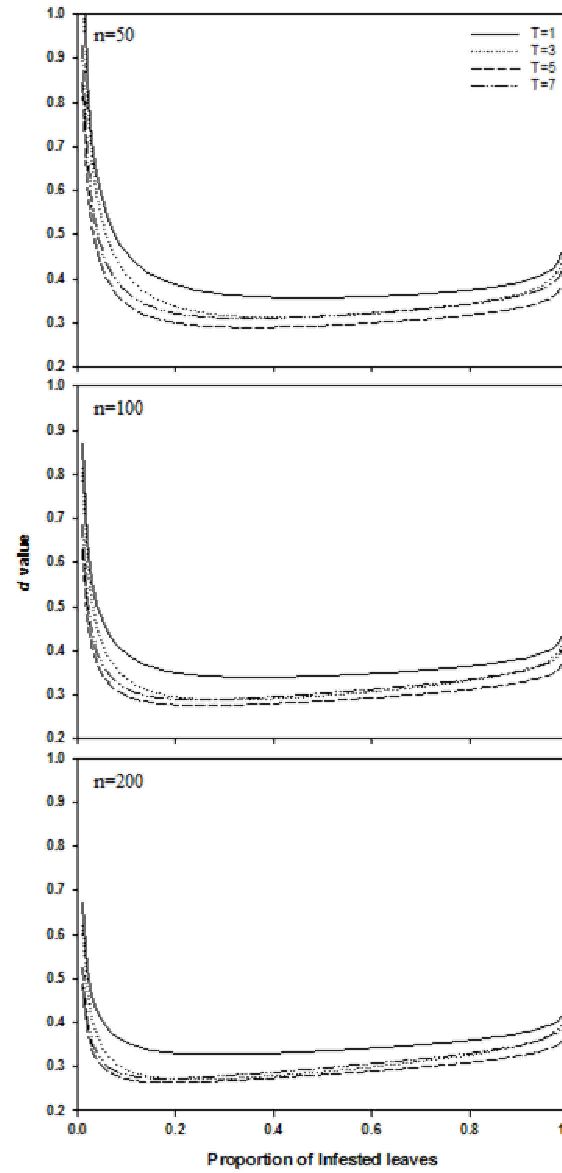

Fig. 4. The sampling precision (*d*) expressed as a function of proportion of infested at the sample size of 50, 100 and 200 (*B. tabaci* pupae).

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. 3, 4), using Eq. (3). Increasing the sample size from 50 to 200 had little effect on the sampling precision, regardless of the tally threshold (Fig. 3, 4). This result is similar to binomial sampling plans of *Thrips palmi* in potatoes (Cho et al., 2000), *Bemisia tabaci* in cotton (Naranjo et al., 1996), *Frankliniella spp.* in tomatoes (Salgyero-Bavas et al., 1994) and *Panonychus citiri* in Satsuma Mandarin groves (Song et al., 2001). This is because sampling size has relatively little weight in the estimation of variance (Jones, 1994). In contrast to sample size, increasing the tally threshold had a dramatic effect on the precision of the binomial sampling plans (Fig. 3, 4). In the case of *B. tabaci* adults, $T = 1$ gave the best precision at all sample sizes. With a sample size of 30, the precision at $T = 1$ was equal to or better than 0.25 for P_T values between 0.23 and 0.95 (Fig. 3). In the case of *B. tabaci* pupae, $T = 5$ gave the best precision among all tally thresholds tested (Fig. 4). Meanwhile, *MSE* is the largest variance component

(Nyrop and Binns, 1991). In this study, the lowest *MSE* values among all tally thresholds were observed at $T = 3$. In order to reduce the effort and time spent on sampling protocols, $T = 3$ was selected as the best tally threshold for *B. tabaci* pupae (Table 1). For the sample size of 200, the precision at $T = 3$ was equal to or better than 0.30 for P_T values between 0.08 and 0.69. Therefore, proper tally thresholds are selected in this study as $T = 1$ for *B. tabaci* adults and $T = 3$ for *B. tabaci* pupae. With *B. tabaci* pupae, regardless of tally threshold or sample size, it was not possible to obtain the level of precision, $d = 0.25$, suggested by Southwood (1978) for damage level assessment in a pest management program. These relatively low precision levels reflect the high variability of binomial sampling plans when compared to absolute counts (Nyrop and Binns, 1991). Other methods proposed to estimate the variance of the binomial empirical model would result in different values, as they place different weight on the individual variance components (Jones, 1994). Regardless of the methods, the binomial sampling plans developed in this study could not provide a precision of mean density better than 0.30 at different tally thresholds or sample sizes. Thus the reliability of the variance estimation methods needs corroboration from field data (Tonhasca *ea al*., 1994).

Validation of the sampling plan

The reliability of binomial sampling plans is based on the accuracy of the relationship between the mean density and the proportion of infested sample units (Naranjo et al., 1996). This relationship was evaluated at $T = 1$ (*B. tabaci* adults) and $T = 3$ (*B. tabaci* pupae). Both binomial models ($T = 1$ and $T = 3$) were adequately described, in that all the independent observations fell within the 95% confidence band around the predicted model (Fig. 5). Comparing the estimated mean and the actual mean using FSS and SPRT showed that the means were very similar (Table 2). Therefore, the binomial sampling plan developed in this study is valid.

The binomial sampling plans presented here should greatly enhance the efficiency of monitoring *B. tabaci* adults and pupae in paprika greenhouses (Naranjo et al., 1996). In this study, the best tally thresholds was $T = 1$ for *B. tabaci* adults and T = 3 for *B. tabaci* pupae. In the case of adults, when $T = 1$,

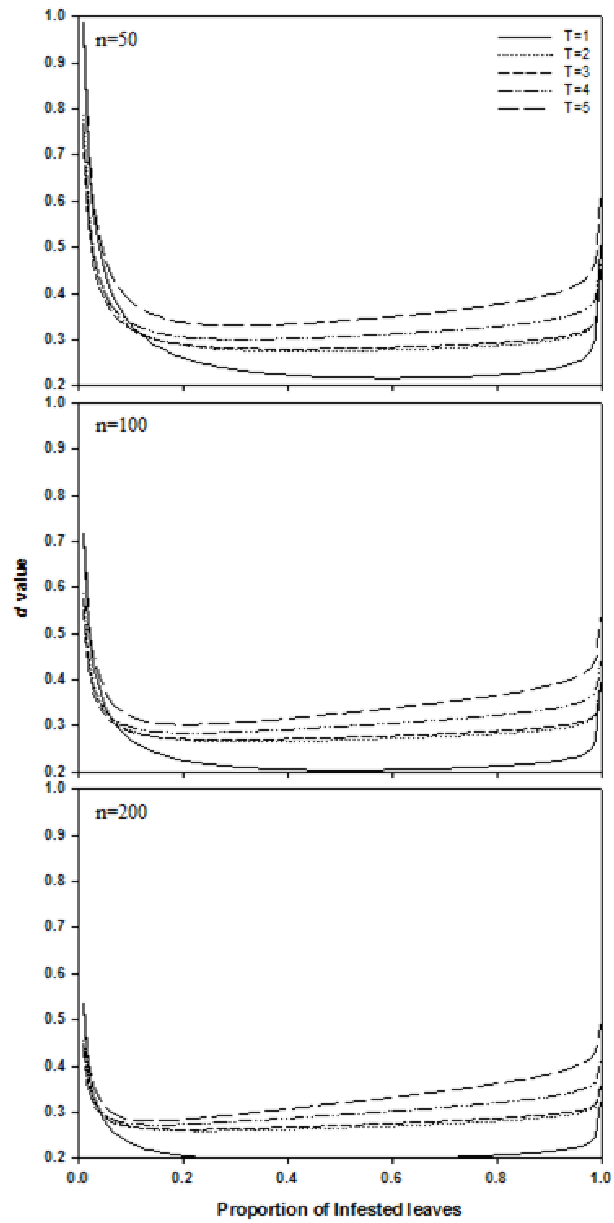

Fig. 5. The fit of the empirical model for independent collected data sets using tally threshold of 1 *B. tabaci* adults and 3 *B. tabaci* pupae per leaf. The dotted lines represent 95% confidence intervals around the predicted equation (middle line)

the r^2 value and the precision were the most suitable, so $T = 1$ was selected as the best tally threshold. On the other hand, in the case of *B. tabaci* pupae, the r^2 value was suitable at $T = 1$ and the precision was appropriate when $T = 5$. However, $T = 3$ was selected as the best tally threshold because *MSE* is the largest variance component (Nyrop and Binns, 1991). Precision for *B. tabaci* adults ($T = 1$) was higher than 0.25 as recommended by Southwood (1978). However, in case of *B.*

Table 2. Resampling validation of fixed-sample-size binomial plan and Wald's sequential binomial plan for independent collected data sets based on binomial counts with the tally threshold of 1 *B. tabaci* adults and pupae per leaf

Stage	Complete count			Average statistics over 500 simulation				
	Date	Density	PI[1]	*n*	Density	PI	ASN[2]	OC[3]
Adults	24 Jan.	0.48	0.22	54	0.48	0.22	19	0.96
	14 Feb.	0.94	0.48	54	0.94	0.48	18	0.02
	21 Mar.	0.83	0.40	54	0.83	0.40	26	0.20
	25 Apr.	0.46	0.27	54	0.46	0.28	26	0.85
	23 May.	0.33	0.25	54	0.33	0.26	25	0.90
	30 May.	0.59	0.51	54	0.59	0.52	15	0.02
Pupae	24 Jan.	0.16	0.01	54	0.16	0.01	20	1
	14 Feb.	0.29	0.03	54	0.30	0.04	20	1
	21 Mar.	0.59	0.05	54	0.60	0.05	20	1
	25 Apr.	6.2	0.40	54	6.2	0.40	35	0.20
	23 May.	3.5	0.25	54	3.5	0.25	29	0.93
	30 May.	1.75	0.10	54	1.76	0.10	20	1

n= Number of sample sizes for each data set
[1] Proportion of infested of leaf
[2] Average sample number
[3] Operating characteristic

tabaci pupae (T = 3), the precision was about 0.3 which is lower than 0.25. This result is believed to be due to the low density of *B. tabaci* pupae on the bottom position because the worker managing the greenhouse removes mostly the leaves at the bottom. Because the life cycle of *B. tabaci* is short and many numbers of individuals are proliferated, the low tally threshold will enable quick decision making and improved efficiency.

Acknowledgement

This study was supported by Rural Development Administration (PJ010851), Korea.

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