Spatial Dispersion and Optimum Sample Size for Cotton Bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) Larvae on Cotton

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**ABSTRACT**

Field experiments were conducted in the irrigated and the rain-fed crops of 2000 and 2001 at Suwan Farm, Pak Chong, Nakon Ratchasima, Northeastern Thailand to determine the spatial dispersion of cotton bollworm, *Helicoverpa armigera* (Hübner) larvae on four cotton varieties/lines, namely, AP1 and AP2, the mutant lines, SR60 (Sri Samrong 60) and SD1 (Sarid1), the recommended varieties. RCB was used with four replicates for both crops. The spatial dispersion was analyzed using the variance-to-mean ratio ($s^2/\bar{x}$), Morisita’s Index ($I_\delta$) and the negative binomial parameter ($k$). Although cotton bollworm larvae exhibited mainly clumped distribution, sometimes it was found to be randomly disperse during the sampling periods of both crops. In general, *H. armigera* larvae were at high aggregation ($k=0.10$) on the irrigated crop and low aggregation ($k=3.92$) on the rain-fed crop. The dispersion information was used to select an optimum sample size at the 0.05 probability. The optimum sample size of 15 and 30 plants per 140 m² were needed to monitor low and high aggregation levels of *H. armigera* larvae, respectively.

**Key words:** *Helicoverpa armigera*, dispersion, cotton, sample size

**INTRODUCTION**

The cotton bollworm, *Helicoverpa armigera* (Hübner), is described as the most destructive and persistant key pest of cotton (*Gossypium* spp.) in Thailand. The larval stage of *H. armigera* causes the greatest amount of damage to cotton with each destroying up to 15 cotton bolls during its development. Seriously damaged cotton fruiting bodies (buds, open flowers and bolls) can be considered as total crop loss, however larvae will eat leaves and young stems when nothing else is available. Consequently, chemical control still remains the backbone of management tactics for *H. armigera*. Before implementing a spray program, pest scouting and economic threshold levels should be determined. In addition, the spatial dispersion of *H. armigera* and optimum sample size should be investigated before sampling decisions are made.

The two most commonly tested mathematical distributions, the Poisson distribution and the negative binomial distribution, are useful for describing random and aggregated distributions, respectively. Dispersion of population can be classified by calculating the indices such as the variance-to-mean ratio ($s^2/\bar{x}$) and Morisita’s Index ($I_\delta$). Variance-to-mean ratio is the simplest and most fundamental index to access an agreement of the data set to the Poisson distribution in which the variance equals the mean (Davis, 1994; Wilson,
1994), while Morisita’s Index has the advantages of being relatively independent type of distribution, the number of samples, and the size of mean (Morisita, 1962; Pieters and Sterling, 1974). Moreover, Morisita (1962) noticed its relationship to the binomial distributions and relative parameter for appropriate samples and stratified random sampling. In general, the values of both indices can be used to estimate whether a populaiotn’s spatial pattern is uniform, random, or aggregated (Pieters and Sterling, 1974; Davis, 1994).

Southwood (1978), Taylor (1984), Perry (1997), and Southwood and Henderson (2000) advocated that analysis of spatial dispersion of insects provides the estimation of pest densities and minimal costs which were the basis for making decision in pest management. Nachapong (1980) and Mabbett and Nachapong (1979, 1983), stated that the egg distribution of cotton bollworm was adequately described by the negative binomial and the spatial distribution remained contagious over the period of sampling. It was also revealed that the taller and healthier plants were more frequented targets for egg laying moths and the eggs were deposited on these plants in higher numbers (Mabbett et al., 1978, 1979). Reliable sampling plans are essential for monitoring pest population densities where timely pest management decision is necessary (Kuno, 1991). Buntin (1994) noticed that stratified random sampling technique could improve sampling efficiency by reducing sample variation that samples were collected from all areas of the habitat. Southwood (1978) and Southwood and Henderson (2000) stated that the number of samples could be varied with the distribution of the insect. Although several studies have been conducted on the dispersion of H. armigera eggs and larvae in Thailand, dispersion of insect may vary with densities and environmental conditions. The objectives of this study were to describe the spatial dispersion of H. armigera larvae and to develop the optimum sample size needed to be taken for desired precision of estimates of an effective sampling program.

**MATERIALS AND METHODS**

Studies were investigated at Suwan Farm (356 m above sea level and 101.25° N, 14.42° E), Pakchong, Nakon Rachasima Province for two growing crops of 2000 and 2001. A field of 0.30 ha was planted with four cotton varieties/lines, namely, Sri Samrong 60 (SR60), Sarid1 (SD1) and new mutant lines AP1 and AP2 of moderate resistance to bollworm, obtained from gamma-irradiated SD1 with a spacing of 1.0×1.0 m. The experiments were arranged in randomized complete block design with four replicates. An individual plot consisted of 7 rows, each 20 m long (140 m²). All varieties/lines were planted on 1 October 2000 for the irrigated crop and 21 July 2001 for the rain-fed crop, respectively. Plots were managed in the same way as commercial cotton planting in this region. Sprinkler irrigation system was applied as required. The field was treated with 38 kg/ha Furadan 3% G and 0.06 kg/ha Imidacloprid 70% WS at planting time of the irrigated and the rain-fed crops, respectively. Carbosulfan 20% EC 50 ml per 20 litres of water and Omethoate 50% SL 40 ml/20 litres of water were alternately applied 5, 6, 7, 8 weeks after sowing (WAS) for the irrigated crop and Azodrin 60% WSC 40 ml per 20 litres of water applied once 4 WAS for the rain-fed crop, to control severe attack by leafhopper.

For the purpose of stratified random sampling, individual plot divided into four strata and 10 plants chosen at random per each plot, totally 160 plants, were visually inspected. H. armigera larvae were counted weekly from 10 to 14 WAS in the irrigated crop and from 7 to 13 WAS in the rain-fed crop, respectively. To analyze the spatial dispersion, means (X) and variances (s²) for counts of larvae per plant were calculated for each sampling date.

Although the variance mean ratio is commonly considered to be a useful index to describe
the spatial dispersion of a given insect, a single index could not serve well both as a measure of departure from randomness or as a measure of aggregation (Kuno, 1991). The two statistical models; Morisita’s Index ($I_\delta$) with $F_0$ value and the negative binomial parameter $k$ were examined to confirm the values of $s^2/x$ ratio (Morisita, 1962; Southwood, 1978; Davis, 1994). The parameter $k$ of the negative binomial distribution is one measure of aggregation that can be used for insect species having clumped or aggregated spatial pattern. As $k$ becomes smaller, the degree of clumping increases; and as the value of $k$ increases, the negative binomial distribution approaches the random Poisson.

The optimum sample size ($n$) was computed to give a reliable estimate. Calculation of optimum sample size depends on the statistical dispersion of the target population and how precision is defined (Southwood, 1978; Southwood and Henderson, 2000). The adjusted sample size ($n'$) is also required to be calculated (Taylor, 1984). The calculations were based on the 4 varieties/lines altogether with 5 sampling dates (10 WAS–14 WAS) for the irrigated and 7 sampling dates (7 WAS–13 WAS) for the rain-fed crops, respectively. The mean temperatures during the irrigated and the rain-fed crops were 25.15°C and 26.02°C, respectively. The total rain-days for the irrigated and the rain-fed crops were 33 days and 75 days, respectively.

**RESULTS AND DISCUSSION**

The dispersion indices ($s^2/x$ and $I_\delta$) of *H. armigera* larvae on four cotton varieties/lines during the 2 years are presented in Tables 1 and 2. All values of both indices were greater than 1 and were confirmed by t-value and $F_0$ value ($p=0.01$), showing that the dispersion of *H. armigera* larvae among cotton plants was clumped. It was found that data of both crops for each cotton variety/line was in consistent with the report that the distribution of cotton bollworm eggs and larva fitted the negative binomial (Nachapong, 1980; Mabbett and Nachapong, 1983; Wilson and Room, 1983). Overdispersion could be apparent when the sample variance was significantly higher than the expected Poisson theory as judged by the index of dispersion (Kuehl and Fye, 1972). The values of negative binomial parameter $k$ were also described ranging from 0.10 to 0.21 in the irrigated and from 2.89 to 9.00 in the rain-fed crops, respectively (Tables 1 and 2). According to Southwood (1978), increasing in the value of $k$ was associated with decreasing tendency for aggregation, whereas the decrease in $k$ value indicated high aggregation at that time. Among the cotton varieties/lines, the highest aggregation was found in SR60 for both crops (Tables 1 and 2). This might be due to the different plant types and moderate resistance to the cotton

<table>
<thead>
<tr>
<th>Variety /line</th>
<th>Variance mean ratio ($S^2/x$)</th>
<th>t-value</th>
<th>Distribution pattern</th>
<th>Morisita’s Index ($I_\delta$)</th>
<th>$F_0$</th>
<th>Distribution pattern</th>
<th>k-value</th>
<th>Distribution pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP1</td>
<td>1.17</td>
<td>17.00</td>
<td>clumped</td>
<td>6.20</td>
<td>1.17</td>
<td>clumped</td>
<td>0.21</td>
<td>clumped</td>
</tr>
<tr>
<td>AP2</td>
<td>1.27</td>
<td>18.62</td>
<td>clumped</td>
<td>7.18</td>
<td>1.27</td>
<td>clumped</td>
<td>0.18</td>
<td>clumped</td>
</tr>
<tr>
<td>SD1</td>
<td>1.37</td>
<td>28.03</td>
<td>clumped</td>
<td>12.44</td>
<td>1.37</td>
<td>clumped</td>
<td>0.10</td>
<td>clumped</td>
</tr>
<tr>
<td>SR60</td>
<td>1.64</td>
<td>46.7</td>
<td>clumped</td>
<td>23.33</td>
<td>1.64</td>
<td>clumped</td>
<td>0.05</td>
<td>clumped</td>
</tr>
</tbody>
</table>

$^\mu$ $P=0.01$
Table 2  Evaluation of spatial dispersion for *Helicoverpa armigera* (Hübner) larvae for four cotton varieties/lines at weekly intervals (7 WAS-13 WAS) during the rain-fed crop.

<table>
<thead>
<tr>
<th>Variety /line</th>
<th>Variance mean ratio $(S^2/\bar{x})$</th>
<th>t-value</th>
<th>Distribution pattern</th>
<th>Morisita’s Index $(I_\delta)$</th>
<th>$F_0$</th>
<th>Distribution pattern</th>
<th>k-value</th>
<th>Distribution pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP1</td>
<td>1.04</td>
<td>1.00</td>
<td>clumped</td>
<td>1.20</td>
<td>1.04</td>
<td>clumped</td>
<td>4.00</td>
<td>clumped</td>
</tr>
<tr>
<td>AP2</td>
<td>1.02</td>
<td>0.67</td>
<td>clumped</td>
<td>1.11</td>
<td>1.02</td>
<td>clumped</td>
<td>9.00</td>
<td>clumped</td>
</tr>
<tr>
<td>SD1</td>
<td>1.07</td>
<td>1.75</td>
<td>clumped</td>
<td>1.40</td>
<td>1.07</td>
<td>clumped</td>
<td>3.00</td>
<td>clumped</td>
</tr>
<tr>
<td>SR60</td>
<td>1.06</td>
<td>2.25</td>
<td>clumped</td>
<td>1.40</td>
<td>1.06</td>
<td>clumped</td>
<td>2.89</td>
<td>clumped</td>
</tr>
</tbody>
</table>

$\frac{\mu}{\nu} \quad P = 0.01$

bollworm of the other varieties/lines.

Table 3 expresses an overall dispersion of *H. armigera* for four cotton varieties/lines. The highly aggregated level ($k=0.10$) in the irrigated crop was observed, while low aggregation ($k=3.92$) fell in the rain-fed crop (Table 3). The low value of $I_\delta$ (1.27) in the rain-fed crop showed that even at a very low density, individuals tended to aggregate. High causes of aggregation ($I_\delta=10.40$) was recorded in the irrigated crop (Table 3). The reason might be due to the heterogeneity of the environment such as microclimate, preferred parts of the plant and occurrence of natural enemies.

For the irrigated crop, fractional values of $k$ were obtained for each sampling date in each cotton variety/line ranging from 0.001 to 0.25 (Figure 1). The peak (0.25) was observed 10 WAS in AP1, then sharply declined until 14 WAS and never exceeded even at a value of 0.50 (Figure 1), showing the bollworm populations to be highly aggregated in this crop. In the rain-fed crop, the individual $k$ values for each sampling date differed considerably, ranging from 0.001 to 4.5 (Figure 2). The peak (4.5) was recorded 7WAS in AP2 and rapidly decreased in later sampling dates (8 WAS to 13 WAS) (Figure 2). The aggregation peaks of both crops might be coincided with the early larval stage of bollworm. The results confirmed the previous findings in revealing that as most population aged they became progressively less clumped (Pieters and Sterling, 1974; Wilson and Room, 1983). Southwood (1978) mentioned that $k$ value could be influenced by predation, size of sampling units and weather. It was reported that the actions of predators and other

Table 3  Summary of spatial dispersion for *Helicoverpa armigera* (Hübner) larvae during the irrigated and the rain-fed crops of 2000 and 2001.

<table>
<thead>
<tr>
<th>Cropping seasons</th>
<th>Variance mean ratio $(S^2/\bar{x})$</th>
<th>t-value</th>
<th>Distribution pattern</th>
<th>Morisita’s Index $(I_\delta)$</th>
<th>$F_0$</th>
<th>Distribution pattern</th>
<th>k-value</th>
<th>Distribution pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigated</td>
<td>1.34</td>
<td>34.00</td>
<td>clumped</td>
<td>10.40</td>
<td>1.34</td>
<td>clumped</td>
<td>0.10</td>
<td>clumped</td>
</tr>
<tr>
<td>Rain-fed</td>
<td>1.05</td>
<td>2.50</td>
<td>clumped</td>
<td>1.27</td>
<td>1.05</td>
<td>clumped</td>
<td>3.92</td>
<td>clumped</td>
</tr>
</tbody>
</table>

$\frac{\mu}{\nu} \quad P = 0.01$
mortality factors could be implicated in the progressive reduction in aggregation of bollworm life stages (Pieters and Sterling, 1974).

The overall dispersion of *H. armigera* at each sampling date in the irrigated crop revealed mostly an aggregated dispersion (Figure 3). However, in a few cases the expected frequencies of the negative binomial dispersion did not fit the observed dispersion. On those occasions, $s^2/\bar{x}$ did not depart significantly from 1. It was observed that larvae were randomly disperse at two sampling dates (10 WAS and 13 WAS) in the irrigated crop (Figure 3). The peak of $s^2/\bar{x}$ (3.4) was recorded 11 WAS (Figure 3). The result was similar to those reported by Kuehl and Fye (1972) and Wilson and Room (1983) that the bollworm larvae tended to be randomly distributed when population density was low.

A similar trend was recorded for the rain-fed crop that samples collected from 7 WAS to 9 WAS (early stages of the sampling period) were found randomly disperse (Figure 3). Thereafter, *H. armigera* larvae exhibited clumped dispersion alternated with random dispersion. The distinct peak of $s^2/\bar{x}$ (2.2) was found 10 WAS (Figure 3). The finding was in line with the report that spatial dispersion of *H. armigera* larvae was random during the early development period of the cotton plants after which they exhibited either random or clumped distribution (Buranapanichpan, 1989). Morisita (1962) and Taylor (1984) stated that change in distribution from clumped to random resulted from the alteration of the size of the area occupied by the insects related to that of the sample or decreased population density. In both crops, there was a significant number of most samples ($s^2/\bar{x} >1$)
expressing agreement with negative binomial dispersion probabilities. Southwood (1978) revealed that when the population of an area became sparse, the chances of an individual occurring in any sample unit was low and continuous, the dispersion was effectively random. He also stated that dispersion might be changed because of the movement of medium and large larvae in some situations. In addition, Pieters and Sterling (1974) reported that cannabalistic behavior of \( H. \) armigera would help reducing aggregation. As different larval instars were present at some sampling dates and so different behaviors exhibited leading to different times of adult emergence, which could result in clumped together with random distribution during the investigation period. Moreover, the cotton field sprayed with some insecticides might affect the level of oviposition and dispersion pattern of \( H. \) armigera larvae. Nachapong (1980) previously stated that the distribution of cotton bollworm was dependent on both egg laying habits of the female moths and variation in growth among the cotton plants.

The information of spatial dispersion could be used to prepare sequential sampling plan of \( H. \) armigera. It was found that stratified random sampling and whole plant visual sampling method could be applied for bollworm scouting program. Minimum 15 cotton plants could be monitored at low aggregation level \((k > 1)\) for 0.05 probability of being within 10% of the mean number of bollworm larvae per plant. This result agreed with Wilson (1994) who reported that a smaller sample size was required at lower population aggregation for a given level of reliability. However, when population was highly aggregated \((k < 1)\), sample size should be increased up to 30 plants \((p=0.05)\) per 140 \( m^2 \).

**CONCLUSION**

The dispersion pattern of \( H. \) armigera larvae among cotton plants changed over the season, showing first an aggregation and subsequently, a random dispersion. The overall pattern of \( H. \) armigera dispersion remained essentially the same whatever the cotton growing crop as well as the cotton variety/line. Random dispersion might be observed at the low population densities expressing consistency with Poisson distribution. Samples obtained from relatively dense insect population were the negative binomial dispersion. The sample size was influenced by the statistical distribution of the samples. It was possible to assume that optimum sample size of 15 plants and a considerably larger sample size of 30 plants per 140 \( m^2 \) were required at low and high aggregations of \( H. \) armigera larvae, respectively. Although the population density should be monitored throughout the season, it is possible that the sampling times and labor costs could be reduced.

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**LITERATURE CITED**


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