SPATIO-TEMPORAL DISTRIBUTION AND ENUMERATIVE SAMPLING OF THE PINK BORER, SESAMIA CRETICA LED. (LEPIDOPTERA: NOCTUIDAE), IN MAIZE FIELDS IN EGYPT

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(Accepted 29 November 2001)

Abstract—Surveys for population densities of the maize borer Sesamia cretica Led. (Lepidoptera: Noctuidae) were conducted for three consecutive years (1997–1999) in several localities of the Nile delta in Egypt, in order to get a better insight into the population dynamics of the pest and to propose rational basis for its biocontrol using a recently isolated granulovirus. High population densities (more than one borer per plant) were observed only in the first plantings after winter (first fortnight of April). After this peak, populations almost vanished until the end of the crop season (September–October) when a slight increase was observed (0.1 borer per plant). The drastic decrease in population densities in plantings from the end of April occurs because most final instars of the borer leave the plant to pupate in the soil. This behaviour, uncommon in maize borers, results in a very high mortality rate due mainly to pupal parasitism. Only the few larvae pupating in the stem, which are much less parasitised, give rise to adults. Spatial distribution was studied using Taylor’s power laws which enabled the elaboration of enumerative sampling plans for eggs, small larvae (1st and 2nd instars) and older ones. Fixed optimal sample size and sequential sampling plans for different precision levels were developed. The precision of the estimated sample size was checked using resampling of some of the data that were not used to build the models. The validation study showed that the plans were suitable for the entire Nile delta region.

Key Words: pink borer, Sesamia cretica, maize, population dynamics, sampling, Egypt

Résumé—Un suivi des densités de population du foreur du maïs Sesamia cretica Led. (Lepidoptera: Noctuidae) a été réalisé durant trois années consécutives (1997–1999) dans plusieurs localités du delta du Nil en Égypte dans le but de mieux comprendre la dynamique des populations du ravageur et de fournir une base rationnelle pour la mise en œuvre d’une méthode de lutte à l’aide d’un granulovirus récemment isolé. De fortes densités de population (plus de 1 foreur par plant) n’ont été observées que dans les cultures semées dès la fin de l’hiver (première quinzaine d’avril). Après ce pic, on observe une quasi-disparition des populations jusqu’à la fin de la saison culturelle (septembre–octobre) où un léger accroissement de densité se produit (0.1 foreur par plant). La chute brutale des densités de population dans les cultures semées à partir de fin avril a pu être expliquée par le fait que la plupart des larves de dernier stade quittent la plante pour se nymphoser dans le sol. Ce comportement, inhabituel chez les foreurs du maïs, a pour conséquence une très forte mortalité due principalement au parasitisme nymphaïal. Le taux de parasitisme des rares larves se nymphosant dans les tiges est...
beaucoup plus faible. Très peu d’adultes émergent, provenant uniquement des larves non parasitées se nymphosant dans la tige. La distribution spatiale du ravageur a été étudiée à l’aide de la loi de puissance de Taylor, qui a permis l’établissement de plans d’échantillonnage énumératifs pour les œufs, les jeunes larves (1er et 2nd stade) et les larves plus âgées. Des tailles d’échantillons optimales pour différents niveaux de précision ont été établies à l’aide de plans d’échantillonnage fixes et séquentiels. La précision de la taille d’échantillon estimée a été testée à l’aide du rééchantillonnage d’une partie des données qui n’avait pas été utilisée pour la construction des modèles. Cette étude de validation a montré que les plans étaient applicables pour l’ensemble du delta du Nil.

Mots Clés: Sesamia cretica, maïs, dynamique des populations, échantillonnage, Égypte

**INTRODUCTION**

In Egypt maize, *Zea mays* (L.), is infested by three borer species: *Chilo agamennon* Blesz. (Lepidoptera: Pyralidae), *Ostrinia nubilalis* Hbn. (Lepidoptera: Pyralidae), and *Sesamia cretica* Led. (Lepidoptera: Noctuidae). The pink borer, *S. cretica*, is a pest of maize and sugarcane mainly in the eastern Mediterranean countries. Its distribution extends eastwards to Iran and southwards to Somalia and Ethiopia (Abul-Nasr, 1977). Females of *S. cretica* lay their eggs under the leaf sheath, and newly hatched larvae enter the plant whorl or stem. In Egypt, population densities of *S. cretica* are high in the Nile delta, especially on early maize crops, sown between late March and mid-May (Mostafa, 1981), in which the borer may cause severe damage (Seemada, 1985, 1988).

Control of the pest in highly infested crops is currently achieved with chemical insecticides. However, the intensive use of insecticides in the Nile delta has produced resistance in many insect species (El-Sayed and Sammour, 1988; El-Sebae et al., 1993). For this reason, as well as environmental and health concerns, including the need to decrease residues in export produce, the Egyptian authorities have forbidden the use of many insecticides in the past few years, supporting, instead, biological control studies for pest management.

Recently, a granulovirus belonging to the family Baculoviridae, was isolated from *S. cretica* in Egypt (Fediere et al., 1993). Granuloviruses are potentially promising agents for the microbiological control of pests, and present no hazards to human health. Experiments were therefore conducted in Egypt to examine the feasibility of using the granulovirus of *S. cretica* (SecrGV) as a biocontrol agent of the borer. At the same time, observations on the spatio-temporal dynamics of the species in the Nile delta were done. The virus treatments resulted in a rapid and highly significant reduction of pest density and proved to be efficient against the borer (Moyal et al., 2001).

In this paper the results of observations on the temporal and spatial changes of *S. cretica* are presented. They give a first basis for a rational pest management using SecrGV by defining the periods of high infestation, and enabling the determination of sample sizes required for estimating insect density at given precision levels.

**MATERIALS AND METHODS**

Collection of data

Samples of maize plants were taken in three localities of the Nile delta, namely Sakha (ca 150 km north of Cairo in 1997, 1998 and 1999), Gemmeza (ca 100 km north of Cairo, in 1998 and 1999), and Kousena (ca 70 km north of Cairo, in 1998); and also in a village located ca 30 km south of Cairo, at Ekhsas, in 1998 and 1999. The climate of the Nile delta is characterised by low rainfall (for instance 50 mm in Sakha in 1998) limited to the winter months. Therefore crops are irrigated. Mean air temperatures vary from a minimum of about 10 °C in January to a maximum of about 35 °C in August. Mean relative air humidity varies between 55 and 70%.

The maize varieties used, SC10 (in 1997) and Giza 2 (in 1998 and 1999), are the most commonly grown in Egypt. Samplings were done either in observation fields (from three weeks after sowing until maturity) or in experimental fields where the virus efficacy was tested (only before treatments were applied). The observation fields were planted at different dates in the year, every two weeks (at Sakha, Gemmeza and Ekhsas in 1999), or every 6 weeks (at Gemmeza and Kousena in 1998), which permitted the study of the population changes throughout these two years. Only early
plantings (April–May) were used for the study of spatial distribution because S. cretica populations reduced drastically in summer, reaching such low densities that they could hardly be detected (see Results). Fields were divided into two to four blocks where weekly samples of 50 to 120 randomly selected plants were taken. Dissection of plants was done in laboratory and the numbers of eggs and larvae were counted.

**Statistical analysis**

Enumerative sampling procedures were developed for eggs, small larvae (first and second instars) and large larvae (older than 3rd instar). First and second instars were considered together because they are not easily distinguished and also because virus treatment is efficient against these stages (Moyal et al., 2002). Moreover, inasmuch as eggs are laid only in the first weeks of the cycle, eggs and the first two instars are frequently found together at the beginning of the cycle, after which only older instars are found.

To calculate the enumerative optimal sample size for a given precision level, the dispersion of the pest was first described using Taylor's power law (TPL) (Taylor, 1961). The law gives a relationship between variance ($s^2$) and mean ($m$): $s^2 = am^b$. The relation between $\ln(s^2)$ and $\ln(m)$ is then linear and can be fitted using simple linear regression. The precision level on $m$ is given by the relative precision

$$C(m) = \sqrt{V(m)}/m,$$

with $V(m)$ being the variance of $m$. By incorporating TPL in this formula it is possible either to get a fixed optimal sample size

$$n = am^{-b}/[C(m)]^2$$

or to build a sequential sampling plan by calculating a stop line (Green, 1970):

$$\ln(T_n) = \ln(C(m)^2/a)/(b-2) + (b-1)\ln(n)/(b-2)$$

where $T_n$ is the cumulative total number of insects and $a$ and $b$ are the coefficients of TPL. Sampling is stopped when the total number of insects found for a given sample size is higher than the expected value from the stop line.

With such a sample size it is then possible to consider that there is some probability that the true population mean lies within a confidence interval of $za/2\sqrt{V(m)}$ ($z_{a/2}$ being the standard normal deviate: for instance, for $P=0.9$, $z_{0.05} = 1.65$) (Karandinos, 1976).

Some of the data were not used to build the models but were kept to validate them (all the samples from Kousens and Ekhsas, and one part of the samples of Sakha 1997, i.e. 25% of the data). Two kinds of validation were done. First, Taylor's power laws were validated by comparing the predictions to actual values, and checking if the slope of the fit was different from 1 (Tomassone et al., 1983). Second, the assumption that the sample size estimated by the model should produce the precision level chosen ($C=0.182$, i.e. give a confidence interval of 0.3 for the mean at a probability level of 0.9), was tested using resampling. The method used here was similar to the one used by Hutchinson et al. (1988) and Naranjo and Hutchinson (1997). For the fixed optimal sample size, the number of plants to be sampled to obtain the required precision level was calculated for each validation sample, and then resampling of the sample with such a number of plants was done. For the sequential sampling plan, resampling was stopped when the cumulative insect number exceeded the expected value from the stop line. The resampling precision level and its confidence interval were then compared to the chosen precision level. The method was then similar to a bootstrap but the sizes of the samples drawn from the data set were not usually equal to the number of observations in the data set. The data sets were resampled 250 times, which is adequate for most situations (Efron and Tibshirani, 1986), and particularly to estimate standard errors (Mathsoft, 1999a).

The software used for these analyses was S-plus 2000 (Mathsoft, 1999b).

**RESULTS AND DISCUSSION**

Changes in population densities throughout the year

The changes in population densities of S. cretica during the year were rather similar in the different locations of the Nile delta and during the different years (Figs 1–3). The first maize cycles, planted during the first fortnight of April, were highly infested. Borer density in these cycles ranged from 1 to 4 per plant. *Sesamia cretica* is a species that
Fig. 1. Changes in larval populations at Sakha in 1998 and 1999

Fig. 2. Changes in larval populations at Gemmeza in 1998 and 1999
hibernates in its last instar. The larval populations of these first cycles resulted from eggs laid by adults formed from the hibernating larvae. In most cases the first planting (beginning of April) was much more infested, and borer density decreased quickly in the following plantings.

The first egg-masses were laid on very young maize plants, often before thinning, which is usually done about 25 days after sowing for these early plantings. For instance, at Sakha in 1998, egg-masses were observed 17 days after sowing, at the maize phenological stage of 4.4 leaves. At Gemmeza in 1999, where maize grew quickly because of high temperatures, many eggs and larvae were found 18 days after planting, at the phenological stage of 5.9 leaves. The presence of young larva at this date indicated that the first egg-layings occurred about one week before, namely only 10 days after sowing. Maize plants were no longer attractive to borers after the phenological stage of about 8 leaves per plant.

After this first generation arising from the hibernating larvae, the borer populations became very low until the end of September, where they increased slightly (reaching about 0.1 larvae per plant) resulting then in the hibernating larvae of the end of the year. This last generation behaved very differently from the larva of the beginning of the year: in fact, they did not infest young maize but were mainly found in maize ears, where many of them hibernated.

This trend of annual population density of S. cretica, which peaked after the hibernation period and then vanished until September, seems to be normal in the Nile delta. Mostafa (1981) observed a similar pattern in 1979 and 1980 at two locations near Cairo: Bahtim (20 km north from Cairo) and Giza, a Cairo suburb.

South of Cairo, however, in the village at Ekhsas, the case was quite different. The population density of S. cretica remained low (less than 0.05 per stem) in all the maize fields planted every two weeks. This corroborates Mostafa's (1981) results of light trap catches of S. cretica adults in Beni-Suef (a region 60 km south of Ekhsas), which were very low throughout the year.

The observed changes in density may be explained by several factors:

**Temperatures at the beginning of spring**

The rate at which hibernation ends depends on temperatures prevalent in March–April. Studies
on insect phenology (Moyal, unpublished data) showed that high temperatures (ca 30 °C) resulted in quick and nearly synchronous adult emergence, whereas lower temperature (ca 20 °C) delayed adult emergence and separated emergence dates. This might explain the case of Sakha in 1999 where the first planting was less infested than the second one. Indeed, temperatures in April 1999 were very low compared to those of 1998 (15 °C versus 23 °C in 1998), which probably delayed and separated emergence.

Rate of egg parasitism

*Sesamia cretica* eggs were parasitised by *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae). However, during the first plantings of the beginning of April the parasitism rate was very low (between 0 and 2 %) during the egg-laying peak (more than 2 eggs per plant), and became high (between 40 and 100 %) only during the last week of oviposition when egg density was low (0.2–0.3 eggs/plant). Therefore egg parasitism was not a factor in greatly reducing population density of the borer during these plantings. In contrast, in the mid-April plantings (for instance the second planting at Gemmeza in 1999), the rate of egg parasitism increased much quicker and this factor then played a greater role in the regulation of borer populations. In later plantings, from mid-May, only a single non-parasitised egg-mass of 22 eggs was recovered from the 100 plants in Gemmeza sown on 10 August 1999.

Survival of larval stages

The rate of survival of newly hatched larvae in natural conditions was estimated in three fields. The number of first instars of a given week was compared to the number of non-parasitised eggs of the previous week. The average survival rate was between 41 and 57 %. Mortality of older instars was lower than that of younger ones. The population density was rather constant for these older larvae throughout the cycle. For instance at Kousena in 1998, 2.1, 3.1 and 4.2 % dead larvae were found in the stems on 18 May, 27 May and 01 June 1998 respectively. This death was apparently caused by bacterial infection. We did not observe symptoms of granulosis. We very rarely observed parasitised larvae, in contrast with Mostafa (1981) who found rates of parasitism of 10–15% throughout the year. Important differences may then exist between regions or years for the level of larval parasitism.

Pupal mortality

Very few pupae were found in the stems in comparison with the larval numbers. Thus at Kousena in 1998, the larval density decreased by 3.1 larvae for every 0.18 pupae found per stem, namely a pupal production rate of only 5.8 %. Similar results were observed at Gemmeza: the rate of pupal production in the stem was 9.2 % (0.12 pupae for a larval density reduction by 1.30) in 1998, and 3.2 % in 1999 (0.15 pupae per stem for a reduction of the larval number by 4.66). The cause of this phenomenon was elucidated: most of the larvae left the plant just before pupation and entered the soil, to pupate within five centimetres into the soil surface.

This behaviour, as far as we know, has never been observed, and is not mentioned in the literature. Moreover no other African borer is known to pupate mainly in the soil. All the other species, including *Sesamia* spp., are known to pupate either in stems or ears or rarely between the leaf sheath and the stem. The behaviour of soil pupation appears then to be peculiar to *S. cretica*. This behaviour is particularly important because it explains the disappearance of the borer populations after the high peak of the first generation. In fact, when they leave the stems, the larvae move from a cool and wet environment (the stem) to a hot and dry one (the soil). For instance in May–June 1997, when the maximum air temperature in Sakha was 29 °C, the maximum soil temperature at a depth of 5 cm was 45 °C. This heat and water stress may be a cause of high mortality. Secondly, pupae in the soil are much more highly parasitised by *Conomorium emerita* (Foerster) (Hymenoptera: Pteromalidae) than pupae found in stems. For instance, the parasitism rate of the pupae found in the stem at Kousena in 1998 was 13.0 %, and no pupa found in the stems at Gemmeza 1999 was parasitised. Mostafa (1981) indicated rates of pupal parasitism between 10 and 30%. Of the pupae collected in the soil at Gemmeza 1999, 78% were parasitised. The other pupae collected in the soil died, either because they were also parasitised but the parasites could not develop and were not detected when the pupae were dissected, or because of other stress factors like heat or dryness. Therefore, only the healthy pupae produced in the stems, which were very few, could emerge into adults.
The low number of adults produced and the dispersion of the emergence times probably resulted in difficulties in mating, which drastically reduced the borer populations. Thus, no egg laying was observed in the second planting at Kousena in 1998, although it was at an attractive phenological stage at the time we observed the emergence in the laboratory, of adults from larvae collected in the first planting. Such a high reduction in population densities had been observed from as early as 1920s (Willocks, 1925; Mostafa, 1981) but until now its causes remained unknown.

Spatial distribution

The fitted models (Taylor’s power laws) are presented in Table 1 for eggs, small larvae (first and second instars) and large larvae (older than 3rd instar). They show good fits to the data with \( R^2 \) close to or higher than 0.9. Most of the densities observed for each of the instar sets studied ranged between 0 and 4 per plant.

Aggregation was very high for eggs. It decreased drastically for young larvae and then continued to reduce, but less quickly from small to large larvae. Thus for instance, with an average of 2 eggs per plant, only 11.7% of the plants were infested, whereas 54.5 and 71.2% of the plants were infested, with 2 small larvae and 2 large larvae per plant, respectively. Larval aggregation in *S. cretica* is similar to that of the species of the same genus that occupies the same ecological niche in countries south of Sahara, *Sesamia calamistis* Hampson. The spatial distribution of this species was studied by Schulthess et al. (1991) for the entire larval stage and, in the range of 0–3 *S. calamistis* larvae per stem, the percentage of plants infested was intermediate between those observed for small and large larvae of *S. cretica*.

Table 2 presents the results of the regressions of the predictions of TPL for the data not used in the models versus the actual values for eggs, small larvae and large larvae, respectively. In all cases the regression slopes were not significantly different from 1, which shows that the models

### Table 1. Fitted models for (a) eggs, (b) small larvae (first and second instars) and (c) large larvae (older than third instar) of the relationship between ln(variance) and ln(mean) (Taylor’s power law)

|        | Value   | Standard error | t value | Pr(>|t|) | R-squared | Residual Standard error | Degrees of freedom |
|--------|---------|----------------|---------|----------|-----------|-------------------------|--------------------|
| **a. Eggs** | Intercept | 2.9680         | 0.0413  | 71.8118  | 0.0000    | 0.9458                 | 0.3347            | 64 |
|         | ln(mean) | 1.2726         | 0.0381  | 33.4257  | 0.0000    | 0.8683                 | 0.5802            |
| **b. Small larvae** | Intercept | 1.9048         | 0.0829  | 22.9832  | 0.0000    | 0.8683                 | 0.5802            | 62 |
|         | ln(mean) | 1.2342         | 0.0611  | 20.2140  | 0.0000    | 0.9317                 | 0.541             |
| **c. Large larvae** | Intercept | 0.9104         | 0.1050  | 8.6676   | 0.0000    | 0.8639                 | 0.5180            | 49 |
|         | ln(mean) | 1.2240         | 0.0474  | 25.8458  | 0.0000    | 0.8404                 | 0.5180            |

### Table 2. Validation of the Taylor’s power law models: regression of the predicted values versus the observed values and test of equality of the slope to 1

| Model   | Coefficient | Value | Standard error | R-squared | Residual standard error | Degrees of freedom | Test of slope=1 | Pr(>|t|) |
|---------|-------------|-------|----------------|-----------|-------------------------|--------------------|----------------|---------|
| Eggs    | Intercept   | 0.130 | 0.115          | 0.898     | 1.224                   | 9                  | 0.7873         |
|         | ln(variance)| 1.03  | 0.116          |           |                         |                    |                |
| Small   | Intercept   | 0.2   | 0.22           | 0.8639    | 3.62                    | 16                 | 0.5180         |
|         | ln(variance)| 0.9385| 0.0931         |           |                         |                    |                |
| Large   | Intercept   | -0.145 | 0.132         | 0.917     | 7.714                   | 20                 | 0.8404         |
|         | ln(mean)    | 1.018 | 0.064          |           |                         |                    |                |
gave reliable predictions. According to the results by Trumble et al. (1989), the transportability of models, particularly TPL, from one location to another or from one year to another is sometimes not possible. In the present case the results showed that the models were suitable for different localities or years inside the Nile delta, which is the most important maize-producing area in Egypt.

Optimal sample size

Figures 4, 5 and 6 present the optimal plant sample sizes for enumerative sampling for different precision levels of the mean number of eggs, small larvae and large larvae, respectively.

The optimal sample size decreased as density increased. In our observations the egg density lay in most cases between 0 and 4 eggs per plant. For such densities, reaching the precision level $C = 0.1$ does not seem to be feasible because of the very high sample size required. The other levels can be used for between 1 and 4 eggs per plant. It is, for instance, necessary to sample between 200 and 300 plants to get a precision of $C = 0.20$ for densities between 4 and 2 eggs. This sample size is still high but for egg estimation, particularly when estimating if a treatment is needed, samples must be taken very early in the maize cycle, preferably before thinning or at thinning; this plant destruction is then not harmful to the crop. For densities lower than 1 egg per plant, the optimal sample size increases very quickly as density decreases and it becomes difficult to get a good precision without large sample sizes. Because of the reduction in aggregation, the sample size for a same precision level decreased for larvae: thus in order to estimate a density of 2 eggs per plant at a precision level of $C = 0.20$, the sample size required is 294 plants, but only 99 plants and 36 plants are required for small and large larvae, respectively. Figure 7 presents the stop lines for sequential sampling, in log-log coordinates, for a $C$ value of 0.182 (i.e. a confidence interval of 0.3 at the level $P = 0.90$). They show clearly the drastic decrease in the sample size required for estimating a given density from eggs to large larvae.

Resampling of the data not used in the models was done for fixed sample size sampling and sequential sampling. In both cases the estimated precision level $C$ was the same, but the standard error of $C$ was much smaller with sequential sampling because the sample size was reduced or increased when necessary according to the population sampled. The comparisons between the resampling estimation of $C$ using sequential sampling (in this case $C = 0.182$) and its confidence interval at $P = 0.05$ with the expected value are presented in Figs 8, 9 and 10 for eggs, small larvae and large larvae, respectively.

For eggs, only two populations in 11 resulted in a slightly higher value of $C$ than the expected
value of 0.182. The other resampled populations resulted in precision levels either not significantly different from the expected value (which was inside the confidence interval) or lower than the expected value. For small larvae, 3 fields out of 18 needed a significantly higher sample size to get the expected precision level. For large larvae, only one estimated C value was higher than the expected among 14 resampled fields. The sample sizes and the stop lines calculated from the models can then be considered as reliable and their use should result, in the great majority of cases, in precision levels in agreement with the expected values.
Fig. 7. Stop lines for sequential enumerative sampling of *S. cretica* at a precision level of C=0.182.

Fig. 8. Validation of the egg enumerative sampling plan: precision levels and their confidence interval estimated by resampling data not included in the model and comparison with the expected value of C=0.182. In abscissa the mean number of eggs per plant.
Fig. 9. Validation of the small larvae enumerative sampling plan: precision levels and their confidence interval estimated by resampling data not included in the model and comparison with the expected value of C=0.182. In abscissa the mean number of small larvae per plant.

Fig. 10. Validation of the large larvae enumerative sampling plan: precision levels and their confidence interval estimated by resampling data not included in the model and comparison with the expected value of C=0.182. In abscissa the mean number of large larvae per plant.
CONCLUSION

The presented results give the prerequisite knowledge for a rational basis of the biological control of the pink borer using SecrGV.

It was found that only the first growing cycles planted at the beginning of spring are highly infested, and therefore biocontrol has to be considered only for these crops. Because pupation occurs mainly in the soil where mortality is high, May plantings are only lightly infested. Understanding the causes of population changes from the observations made could enable their prediction after further studies on modelling of the population dynamics.

The obtained sampling plans allow the estimation of populations of S. cretica with a desired precision in maize fields in Egypt using enumerative sampling. Further studies, including economic aspects, have now to be carried out to finalize the practical strategies of the biocontrol of the pest. The cost of industrial production of the virus must be estimated and the cost of treatments compared to crop losses to define economic thresholds. Models of crop losses due to S. cretica are still lacking and studies on this topic are required. Experiments on the control of the pest using the virus have shown that crop losses were high in the first growing cycles and that the virus was very efficient in the field (SecrGV production from 20 larvae was enough to protect 1 ha) (Moyal et al., 2002), so it is likely that the use of this control method should be economically beneficial.

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