CHAPTER THREE

Materials and Methods

This research work (1994-1995) was divided into two parts, laboratory and field experiments.

3.1 Laboratory Experiments

3.1.1 Stock Culture of *Ostrinia furnacalis* (Guenee)

Adult moths were obtained from field collected the parasitic-free overwintered larvae in cornstalks and in corn cobs in Yongde, Yunnan. These were made at frequent intervals from February through April 1995. The cornstalks and corn cobs bearing overwintered larvae were placed in a aluminum 40 cm × 40 cm × 60 cm cage. The cornstalks bearing overwintered larvae were cut into the short bars about 15 cm short bars, then, put these short corn bars into one cage. The cages containing these plant materials were kept under a constant temperature growth chamber maintained at 25 ± 0.5° C and 70 ± 4% with a light-dark photophase of 16 : 8 hours until adult emergence. Adult moths were transferred into a wooden cage with 30 cm wide, 30 cm deep, and 30 cm high. A piece of waxed-paper was placed inside of the cage for oviposition. Moths were supplied with diluted honey for adult food. They were transferred to fresh cages at each 24 hours interval until ceased laying eggs.

The waxed-paper bearing 1-2 egg masses was cut in a wide 3 cm by 5 cm long. 50 eggs adhering to the waxed-paper was placed in a tray containing 1.5 liters of 0.1% sodium hypochlorite for 10 minutes. The moth eggs were then washed with tap water for 3 minutes and placed in a 1.5 cm disposable Petri dish (100 × 15 standard). Each Petri dish containing 50 eggs were incubated into the growth chamber (LH - 200 - RDCT) under the same conditions as mentioned above until egg hatching.

Newly hatched larvae were transferred from the Petri dish with a wet camel's hair brush onto an artificial diet which was placed in a 10 × 25 cm vial. This media was developed by Zhou et al. (1980) (soybean meal 15g; corn meal 19g; Brewer's yeast 9g; glucose with vitamin mixtures 0.75g; ascorbic acid 0.5g; agar 2g; Formalin 0.2 ml; sorbic acid 0.5g; water 120 ml.). The artificial diet was cut as " # " strips to reduce the chance for larval contact and aggression so that avoid to possible cannibalism. A piece of cotton was used to close the open end of the vial to confine the larvae and to lessen desiccation. Each vial contained 20 larvae. The vials containing larvae were placed on the wooden rack. The wooden racks with the vials were then placed in the propagation chamber and maintained at 25 ± 0.5°C and 70 ± 4% with a light-dark periodphase of 16 : 8 hours until pupae were formed. The pupae were transferred into a plastic sieve. They were surface - disinfected in 0.1% sodium hypochlorite solution and then washed with running tap water for 3 minutes. The pupae were transferred into a clean glass-jar. Each jar, containing moth pupae, was placed in a cage. The cage was kept in a temperature control chamber and left undisturbed until adult emergence. Using this technique, sufficient numbers of moths and eggs were available throughout the study.

3.1.2 The Effect of Temperature on the Duration of Development of *Ostrinia furnacalis* (Guenee)

This test was designed to determine the optimum temperature for development by the Asian corn borer, *O. furnacalis*.

The following assumptions modified from Titayavan (person. conv. 1995) were used to develop the growth model of *O. furnacalis*.

1. All of factors except temperature were kept near optimum conditions for *O. furnacalis* development. Thus, growth patterns were not affected significantly by the other environmental conditions.

2. The response of *O. furnacalis* to temperatures follows a threshold response curve in a sigmoidal function.

3. Observations must be made starting from a temperature at which no growth occurs and extend to an upper limit temperature.

Thus, there will be certain critical parameters which relate to the development of *O. furnacalis*.

a. The lower limit temperature below where no appreciable growth is evident.

b. The upper limit temperature beyond which the rate of development fails to increase with increasing temperatures.

c. The number of heat units (degree-days) required to complete each life stage of *O. furnacalis*.

Nine different constant temperatures were selected for this experiment: 10, 12, 14, 16, 18, 22, 26, 30, and 34°C. A relative humidity 70 ± 4% and a light-dark photophase of 16 : 8 hours were provided in each temperature treatment. 50 less than 24 hour old eggs were introduced into a 10 × 15 cm disposable Petri dish. Four replicates were run for each treatment with total 200 eggs throughout the study. Each Petri dish was removed singly every 24 hour for examination. Daily observations including to monitor survival numbers and time of ecolsion in each event were continued until the moth reached the adult stage or the immatured died. The number of days required for each stage of development from egg to adult emergence was recorded. The theoretical threshold temperatures and degree-days were determined by these data derived from the days of development.

Statistical analysis of the data from this experiment was by analysis of variance and the F-test.

The equation as suggested by Stinner et al. (1974) was used to estimate sigmoid functions and determine the upper threshold temperatures in each life stage of *O. furnacalis*:

$$R_t = \frac{C}{1+e^{k_1+k_2T'}}$$

where:

R_t = rate of development (1/time) at temperature T

C = (maximum developmental rate) × $e^{k_1+k_2 topt}$, i.e. the asymptote

k_1, k_2 = empirical constants

$T' = T$, for $T \leq$ topt

$T' = 2 \times$ topt - T, for T > topt.

topt = temperature at which the maximum developmental rate occurs

SYSTAT Pro (SYSTAT Inc. 1990) was used to estimate the parameters. In this formula, topt was employed as upper threshold (Stinner et al. 1974, Calvin et al. 1991).

The linear regression technique was employed by using the growth rate data as the dependent variable (y-axis) and constant temperatures as the independent variable (x-axis). The minimum developmental threshold temperature was determined as the x-intercept of the linear equation relating temperature to growth rate (Pedigo 1989) from the statistical program in Quatro-Pro (Borland, Scott's Valley, CA).

The equation suggested by Lin et al. (1954) was used to calculate degree-day requirements as:

$$K = d_i\left(t_i - a\right)$$

where K is the thermal constants (degree-days); t_i is the temperatures of incubation, d_i is the mean number of days in incubation at the *ith* temperature, and a is the minimum developmental threshold temperature.

The duration of development was approximated by

$$z = a/(T - x)$$

where z is the developmental time; a is the average degree-day requirements; T is temperature, and x is the lower developmental threshold (Miller and Paustian 1992). By this procedure, the growth model for this moth was constructed and its growth rate calculated.

3.1.3 Life Table Study of *Ostrinia furnacalis* (Guenee)

The life table of Asian corn borer developed from the experiment of the effect of temperature on the duration of development as mentioned as above. That is, 200 less than 24 hour old eggs were placed in each growth chamber of 10, 12, 14, 16, 18 22, 26, 30, and 34°C, respectively. And a relative humidity 70 ± 4% and a light-dark photophase of 16 : 8 hours were provided in each temperature treatment. Observations were made at 24 hour interval until all insects had matured or died. The number of individuals either alive or dead during each age interval was recorded. The life table was constructed with the headings modified by Titayavan (1986). The initial lx was based on the total number 200 eggs. The subsequent mortality of each stage was calculated as k-value, expressed logarithmically was obtained by subtracting of the logarithm of the population after mortality occurred from the logarithm of the preceding population. The summation of k-value yielded the generation mortality.

3.2. Field Experiments

In early May 1995, the field investigations were constructed in an unsprayed corn field in one hectare consisting of 45,000 corn plants at Yongde, Yunnan (1606.2 m altitude, and 24°02' N). The Chinese improved corn hybrid YEDAN-12 variety was used. No insecticide was applied to study plots or any areas nearby. Farm manure was applied two times: the first applying was before seeding as base fertilizer, ca. 4,500 kg / hectare; the second applying was at before tassling stage as topdressing, ca. 3,000 kg / hectare.

3.2.1 Response of *Ostrinia furnacalis* (Guenee) to the Light Trap

Since the adults of Asian corn borer are attracted by light, relative measurements of population of this moth can be obtained by the light traps. One 15-watt blacklight fluorescent lamp trap was placed in the vicinity of experimental corn fields as to minimize interference from the big trees and buildings. The trap was placed at approximately 500 m apart from the pheromone traps at 2m above the ground. The adult moth attracted by the light were diverted by a series of baffles, and funneled into the dichlorvos - impregnated resin strip killing jar at the bottom of the trap. The number of moths of Asian corn borer responding to the trap was counted every 24 hours from early May to early October, 1995.

3.2.2 Response of *Ostrinia furnacalis* (Guenee) to the Synthetic Sex Pheromone

Field testing of the Asian corn borer pheromone was conducted in a corn field consisting of 45,000 untreated corn plants. Traps were constructed from plastic container which was 90 cm diameter rim, 65 cm diameter base, and 35 cm depth containing 5.0 liter water. The common commercial detergent (15 ml.) was added to the water to increase the ability of moth drowning. The synthetic sex pheromone used in this study was plastic cap (JINTAN Inc.). The chemicals were released from a 1.5 × 0.6 cm plastic cap impregnated with this synthetic sex pheromone (E)-12-Tetradecenyl acetate 100 μg. Pheromone caps were held over the water container with a wire loop. Two traps were used in the corn field with being placed at approximately 30 meters apart in the same area. These two traps were examined at 24 hours intervals from early May to early October, 1995 and the numbers of male moth responding to the pheromone were recorded each day. Fresh synthetic sex pheromone were replaced once a week. Each trap was suspended by a small wire hanger 1.5 m aboveground. Traps were maintained on this schedule until male flight stopped during early October.

3.2.3 Sampling for Immature Populations of *Ostrinia furnacalis* (Guenee)

Field sampling for immature stages was conducted in a 96 m^2 block consisting of 450 corn plants. Fifteen time periods from May 26 to September 7, 1995 were used for sampling of Asian corn borer. At the beginning of the whorl stage 60 corn plants were sampled at random from the center of the block. Visual searches for eggs was made by gently turned the leaves ventral side up. Leave in a whorl, and ears were carefully examined as larvae fed within. Plants were dissected carefully with a dissecting knife in the field and examined for moth larvae and pupae. Five replications were run with a total of 300 corn plants sampled. Larvae were divided into three size categories rather than actual instars: small, < 6 mm (first and second instars); medium, 6-12 mm (third and fourth instars); large (fifth instar), > 12mm. Observations were made once a week in order to monitor the development of moths closely under field conditions.

The number of all immature stages was recorded. The spatial distribution was calculated. Spatial distribution pattern was determined by Poisson probability distribution model (Din 1994), negative binomial probability model (Ludwig and Reynolds 1988), and Taylor's power law (Taylor 1961).

The function of Poisson probability distribution model is:

$$P_x = \frac{m^x e^{-m}}{x!}$$

where x is the number of individuals per plant; m is the mean number of individuals per plant. e is the base of the natural logarithms. The symbol $x!$ stands for x factorial.

A Chi-square (χ^2) goodness-of-fit test was used to evaluate how well the selected discrete distribution fits the data as suggested by Sokl and Rohlf (1981). The Chi-square (χ^2) goodness-of-fit test was calculated as:

$$X^2 = \sum_{x=0}^{n} \frac{(O-E)^2}{E}$$

where O is the observed frequency of x, E is the expected frequency and n is the number of frequency groups.

The negative binomial probability model describes an aggregated population by two parameters, the mean m and an aggregation parameter k. The function is:

$$P_x = [m/(m+k)]^x \{(k+x-1)!/[x!(k-1)]!\}[1+(m/k)]^{-k}$$

where x is the number of individuals per plant; m is the mean number of individuals per plant. k is a parameter of degree of clumping; x, m and testing the agreement of an observed frequency distribution with negative binomial are similar to those description as Poisson series.

The estimate of k use the following iterative equation:

$$\log(N/N_0) = k\log(1+m/k)$$

where N is the total number of sample units in the sample, and N_0 is the number of sample units with 0 individuals. As initial estimate of k is substituted into the right-hand side of the equation and the resultant value is compared to the value of the left-hand side. If the right-hand side is lower than the left-hand side, a higher value of k is then tried, and, again, the two sides are compared. This process is continued by selecting higher or lower values of k until a value of k is found such that the right-hand side converge to the same value as the left-hand side. A good initial estimate of k is obtained from the formula suggested by Horn (1988):

$$k = \frac{m^2}{(s^2 - m)}$$

Frequency classes were pooled when the lowest expected values were > 1 (Ludwig and Reynolds 1988).

Taylor's power law related the variance (s^2) to the mean (m) by $s^2 = am^b$. To estimate a and b, the values of ln (s^2) were regressed against those of ln(m) using the model:

$$\ln(s^2) = \ln(a) + b\ln(m)$$

The slope b indicates a uniform, random, and aggregated distribution when $b < 1$, $b = 1$, and $b > 1$, respectively. The hypothesis, H_0: $b = 1$, was analyzed using t-test (Sokal and Rohlf 1981).

For the series of investigation to combine sampling dates, the combined mean and variance were the important factors to determine the spatial distribution patterns. The mean and variance in the combined sampling dates were calculated suggested by Sachs (1984).

$$\overline{X}_{comb} = \frac{n_1 \times \overline{x}_1 + n_2 \times \overline{x}_2 + \ldots + n_i \times \overline{x}_i}{n}$$

where $\overline{x}_{comb}$ is the combined mean; n_i is sample size; $\overline{x}_i$ is mean values in *ith* sample size; n is a single sequence of size and $n = n_1 + n_2 + \ldots + n_i$.

$$s^2_{comb} = \frac{1}{n}\left[\sum_i (n_i - 1)s_i^2 + \sum_i n_i(\overline{x}_i - \overline{x})^2\right]$$

where s_{comb}^2 is the combined variance; n_i is sample size; $\bar{x}_i$ is mean values in *ith* sample size; n is a single sequence of size and $n = n_1 + n_2 + \ldots + n_i$. s_i^2 is the variance values in *ith* sample size.

3.2.4 Arthropod Population Density Assessment

Three time periods for sampling above and under ground arthropod populations as suggested by Hou et al. (1984) were used to evaluate distribution throughout all plant growth periods and relationships among species diversity of all arthropods. These time periods were: (1) corn seedling, about 20 days after planting; (2) the whorl stage, about 60 days after planting; and (3) the physiological mature period, about 100 days after planting. 300 corn plants were randomly selected at each time of three investigation periods.

Direct observation sampling method was used to sample arthropod fauna on corn from seedling through physiological maturity without removal of individuals in the population in the various strata of the canopy. The insects which lived in underground were investigated with limiting scope, that is, the insect species up to shallow layer of soil were included. These underground insect species were collected by handpicking and handsorting with 15 cm depth, and the vicinity of corn plant with a radius 50 cm.

The specimens were identified by species, counted and recorded in the field. Individuals, those which could not be identified were collected for later identification. Identifications were made by Department of Entomology of Yunnan Agricultural University.

The richness used Margalef's index (Magurran 1988) to describe the total species with the total number:

$$D = (S - 1) / \ln N$$

where D is Margurran's index, S is the number of species recorded, and N is the total number of individuals summed over all S species.

Distribution among species was measured by the Shannon index of diversity (H') and index of evenness (E) (Modified Hill's ratio) as suggested by Vandermeer (1981) and Magurran (1988). The Shannon function can be calculated as

$$H' = -\sum_{i=1}^{s^*}(p_i \ln p_i)$$

where H' is the average uncertainty per species in an infinite community made up S^* species with known proportional abundance $p_1, p_2, p_3, ..., p_{S^*}$. H' is estimated from a sample as:

$$\hat{H}' = -\sum_{i=1}^{s}\left[\left(\frac{n_i}{n}\right) \ln\left(\frac{n_i}{n}\right)\right]$$

where n_i is the number of individuals belonging to the *ith* S species in the sample and n is the total number of individuals in the sample.

Modified Hill's ratio is

$$E = \frac{(1/\lambda) - 1}{e^{H'} - 1}$$

where λ is Simpson's index, which is defined as:

$$\lambda = \sum_{i=1}^{S} p_i^2$$

where p_i is the proportional abundance of the *ith* species. λ is estimated unbiased estimation ($\hat{\lambda}$) for sampling from a infinite population as:

$$\hat{\lambda} = \sum_{i=1}^{S} \frac{n_i (n_i - 1)}{n(n-1)}$$

where n_i is the number of individuals of the *ith* species and n is the total number of individuals in the sample. *H'* in the modified Hill's ratio is Shannon's index.

3.2.5 Degree-Day Accumulations under Field Conditions

Temperature data (minimum and maximum) were collected from recording thermographs of Weather Bureau Station of Yongde, located in the field.

For the sine wave methods as suggested by Allen (1976) was used to calculate degree-days. The six possible events as follows:

(1) daily minimum ≥ developmental maximum;

(2) daily maximum ≤ developmental minimum;

(3) daily minimum ≥ developmental minimum and daily maximum ≤ developmental maximum;

(4) daily minimum < developmental minimum and developmental minimum < daily maximum ≤ developmental minimum;

(5) daily maximum > developmental maximum and developmental maximum > daily minimum ≥ developmental minimum; and

(6) daily minimum < developmental minimum and daily maximum >developmental maximum

It was calculated by using a computer program to measure the area under a single sine curve fitted to the day's minimum and maximum air temperatures. Accumulation of degree-days started with January 1, 1995.