CHAPTER III

MATERIALS AND METHODS

1. General:

This work was started with the nymphs as well as the adults of *Oxya nitidula* Walker that were collected from the farmers' fields in Dasuya and Mukerian tract of the district of Hoshiarpur. They were kept in batches of 45-50, males and females together, in laboratory cages (2½' x 1½' x 1½') and were fed on green leaves of *berseem*, *jowar* and maize according to the season. A layer of three-inch deep sand, kept moist by sprinkling water daily over it, was placed at the bottom of every cage. The female grasshoppers laid eggs freely on the green leaves as well as in the moist sand. Most of the eggs used during the experiments were laid by the females reared in the laboratory itself. But, sometimes, when these eggs were not sufficient to meet the requirements of an experiment, mature adults were collected from the experimental fields in Dasuya tract and were left in the laboratory cages for oviposition.

2. Life history at room temperature:

Egg clusters laid within the previous twelve hour periods were collected twice daily at 7.00 and 19.00 hours and were kept in plastic petri dishes (4" diameter), having moist sand at their bottoms, for their embryonic development. The nymphs, on their emergence, were kept individually in wire gauze tubes (4"xl") in order to study their post embryonic development. The nymphs were fed on maize or *berseem*, depending upon the season. Their
food was changed daily; but during the summer months, the particularly before/onset of the monsoon rains, this food was changed twice or thrice daily, as the food plants used would go dry quickly due to the extreme drought of the atmosphere. The matured adults were kept in pairs under glass chimneys (4" diameter) placed on flower-pots (9" diameter) filled with moist sand for oviposition.

3. **Biology under controlled conditions:**

The *Oxya nitidula* was reared throughout its life cycle, i.e., from the egg to the adult stage, under controlled conditions of temperature and relative humidity. Temperature was controlled in the incubators, and was maintained constantly at a point, changing within ±0.2°C only; while the desiccators (4-6 inches) were used for maintaining different, set levels of relative humidity. Calculated densities of sulphuric acid gave the required percentages of relative humidity. The density of the acid was calculated with the following formula:

$$\text{Relative humidity} = \frac{\text{vapour pressure of acid}}{\text{vapour pressure of water}}$$

at a particular temperature.

4. **Lethal temperature:**

Nymphs and adults for this experiment were collected from the fields and were exposed to constant temperatures of 2°C, 5°C, 10°C, 15°C, and 20°C. Fifty individuals in a wire-gauge tube were kept at each constant temperature ± 0.2°C in incubators. The mortality caused was recorded after every six hours.
5. Soil moisture and its effect on the duration and viability of eggs:

Five newly 12 hours laid egg clusters were kept in plastic petri dishes (4" diameter) in which one centimeter deep layer of oven-dried sand was spread. Different levels of soil moisture (by weight) were prepared by adding known quantities of water to the sand. Subsequently, the petri dishes were placed in desiccators having water at their bottoms. It was established in a separate experiment that oven dried sand, when kept for four days in a desiccator having water at the bottom, absorbed 0.25% of moisture by weight. The desiccators, throughout the experimental period were kept in incubators run at a constant temperature of 15°, 20°, 25°, 30°, 35° and 40°C. There were nine levels of soil moisture viz. oven dried sand 0, 1, 2, 3, 4, 5, 10, 15 and 20% with four repeats. The observations were recorded at 7.00 and 19.00 hours.

6. Effect of desiccation on the duration of and viability of eggs:

The effect of desiccation on the eggs was studied by keeping the egg-clusters in oven-dried sand (0% moisture) for different durations. After keeping the egg-clusters for a definite period under desiccation, they were transferred to an optimum moisture, i.e., 10%. There were, in all, fifteen sets used in the experiment. In the first set the egg clusters, immediately after they were laid, were placed under conditions of desiccation and further the period of desiccation ranged from 1-14 days, with an
interval of one day between. As the period of desiccation was over, the egg clusters were kept in optimum moisture. In the second set, the egg clusters were first kept in optimum moisture for one day and after that they were transferred to zero percent moisture level and the duration of desiccation again ranged from 1-14 days after which the egg clusters were again brought to a normal moisture level. Similarly, in all the other 13 sets the first exposure to optimum moisture was made to vary from 2 to 14 days and after which the period of desiccation was repeated in a similar manner. But in the last set, i.e., the sixteenth, the egg clusters were kept in dry sand (i.e., 0% moisture) throughout the experimental period. Efforts were made to collect the eggs for the experiment from the females of the same age. The whole experiment was conducted at a constant temperature of 30 ± 0.2°C in an incubator.

7. Age of the female and its effect on the duration and viability of the eggs:

A set of 20 females of the same age was kept under observation and their egg clusters were collected twice daily, i.e., at 7.00 and 19.00 hours. The egg clusters were kept separately in plastic petri dishes, containing moist sand for embryonic development at 30°C. The emergence of the nymphs from the experimental eggs was recorded twice daily. The entire oviposition span of the experimental females, which ranged from 15th to 50th day of their adult life, was divided into eight periods each being of five days duration viz. 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49 and 50 and above, and the average duration
and viability percentage of the eggs falling under each period was worked out.

II. Behaviour studies:

1. Oviposition behaviour:

(a) Thirty pairs of adults, immediately after their emergence, were kept under glass chimneys, in the month of June, and were offered three types of oviposition sites, viz., hard surface, i.e., wood, hard surface plus leaves and soil plus leaves, to know their preference for oviposition. The first set had in it 10 pairs that were offered hard surface for oviposition. These individuals of the first set were fed at a six-hour interval and for one hour, after which the leaves were removed. In the second set the plant leaves were left intact throughout the experimental period and the third set of 10 pairs were given moist sand and green leaves for their oviposition site preference. This experiment was repeated in the month of September for their second generation.

(b) Another experiment was arranged in a field cage (9'x9'x6'), in the month of May, with the first generation adults, and a similar experiment was repeated in September of the same year with the second generation adults. Four different oviposition sites, viz., soil, plants, dry twigs and dry leaves were placed at random with four repeats in a field cage. Fifty pairs of adults were liberated during each experiment and the egg clusters were collected after four weeks.
(c) The local sugarcane and rice fields were searched for locating egg clusters of the first and the second generation adults in the months of June and October of the year respectively. In an area of one acre, 112 plots, of two feet square each, were taken. The distance from plot to plot was 15 feet and from line to line was 25 feet. The number of egg clusters, both on the leaves of the plants and in the soil, was recorded. Maximum and minimum temperatures and soil moisture of the fields during both the oviposition periods were also recorded.

(d) A set of ten pairs, immediately after their emergence, were kept under netting cloth bags, 3 ft. high, supported by iron frames which were fitted to the wooden pots, (2' x 1'), in the month of June and again in the month of September. Three levels of soil moisture, viz., dry (0.5 to 2%), medium (10 to 15%) and high (standing water) were maintained in each wooden pot. Green maize plants were kept standing in all the three sub-plots in a wooden pot throughout the experimental period. There were five repeats. In another similar set of experiments three soil moisture levels were maintained in three separate wooden pots, all the other conditions being similar.

(e) The nymphs were reared throughout their nymphal stage at constant temperatures of 25°, 30° and 35°C respectively. When their final moult was over, the adults at each temperature were divided into three sets and each set of five pairs was, in turn, placed at all the three temperatures mentioned above in wire gauge tubes (4"x2"), in which moist sand was kept at the bottom. They
were fed on green maize leaves. The observations regarding the amount of oviposition were recorded at 7 A.M. daily.

2. Dispersal in field at different times of the day:

The dispersal of *Oxya* within the field was correlated with the time of the day by collecting grasshoppers with a handnet (handle 4½") at different intervals. The collection of the grasshoppers with the hand-net was started early in the morning before the activity of the grasshoppers had started, i.e., at 4.30 A.M. and at 7.00 A.M., till late in the evening when the grasshoppers became inactive, i.e., at 19.30 and 17.00 in the summer and the autumn months respectively, at an interval of 3 hours each. The grasshoppers, collected in each sweep, were liberated after counting at the place of the collection. During each interval ten sweeps with the hand-net were taken from four different vegetations, viz., *berseem* (1½'-2' height), sugarcane (3'-4'), tall weeds (1½') and *khabbal* grass (4"-6") in May and from three types of vegetation, i.e., rice (3½'), tall weeds (1½') and *khabbal* grass (4"-6") in October. The whole set of these experiments was repeated for ten days in May and for five days in October of that year. Meteorological observations regarding temperature, soil temperature and relative humidity were recorded during each interval before operating the handnet.

3. Daily movement of grasshoppers in the field:

An experiment was made to study the daily or small scale movements of the surface grasshoppers in the field. Two hundred 5th instar hoppers and adults were marked red with artist's oil-paint and were liberated in a
field cage (9'x9'x6'), in which 18-23 cm. high berseem was growing in one half of the area while in the other half, berseem had been cut at the ground level. The movement of the individuals was recorded at fifteen minutes interval, starting early in the morning before they were active, i.e. at 5.00 hours, till they stopped their movements for night rest, i.e., at 19.00 hours. At the same time data on weather and microclimate were collected. Air temperature and relative humidity were recorded with an ordinary dry-and-wet-bulb thermometer and soil temperature was recorded at a depth of 2 cm., by means of a soil thermometer. Light intensity was measured with 'Weston' exposure meter, which is graduated in candles/ft.\(^2\).

Observations regarding air temperature, relative humidity, soil temperature and light intensity were recorded both from within the crop as well as from the Stevenson screen.

4. **Number of Generations:**

The maximum number of generations that could be bred in a year were worked out in the laboratory. The experiment was started with the first batch of nymphs which emerged from the overwintering eggs. For the second and the subsequent broods, the earliest laid egg clusters were taken. Some of the eggs laid by the females of the second generation undergo hibernation while the others hatch in September-November. Therefore, the females of the second generation produced both the first as well as the third generation of the insect. The following experiments were made to assess the ratio of the overwintering eggs forming the nucleus of the first generation, to the eggs which
hatched during September–November and gave rise to the third generation.

(a) The matured adults were kept in field cages in September, 1959, throughout their oviposition period when the emergence of the third generation nymphs and the life of the second generation adults was over, i.e., towards the end of December. Then the egg clusters, along with the empty egg shells, were collected from the soil and the number of the eggs that hatched during October to November, was recorded. The experiment was repeated in 1960–1961.

(b) Ten pairs of the second generation adults, immediately after their emergence, were kept in ten laboratory cages (1'xl'xl') separately. The eggs that were laid daily, were kept separately under natural conditions in order to study their embryonic development. The number of eggs that hatched during prewinter months and that which overwintered, were recorded.

(c) In another experiment the matured adults were kept in one field-cage for only fifteen days and after that all the adults were shifted to a second cage from where they were shifted to the third cage after fifteen days and so on. In this way, the egg clusters of fifteen days' duration were kept separately in different field cages. The percentage of the eggs that hatched during each fortnight interval, was worked out. The meteorological observations regarding temperature, soil temperature, soil moisture and relative humidity were also recorded.
(d) After the prewinter hatching of the eggs laid by the females of the second generation, was over, the egg clusters were collected from the field in December and the ratio of the eggs hatched to that of the overwintering eggs, was worked out.

III. Population studies of *Oxya*:

1. Characteristics of *Oxya* habitat:

*Oxya* spp. is met with all over the State of Punjab. However, in the districts of Kangra, Gurdaspur, Amritsar, Jullundur and Hoshiarpur, the pest appears in the form of a serious pest periodically. Of these districts also certain riverine tracts are liable to heavy infestation while the uplands remain almost free from the species. The riverine tracts locally called *bet* lie along the banks of the rivers and often get flooded with water by heavy rains. It is low lying and has a vegetation peculiar to itself. The soil remains wet for most of the year, which results in its being covered with various species of grasses, hence large tracts of land remain uncultivated. The epidemic comes in cycles, once the pest population assumes a serious status, it usually lasts for 3-4 years. Apparently, the pest build-up seems to have close relationship with the environment. The pest was noted to be more serious in the Dasuya tract of Hoshiarpur district. This area was, therefore, selected for carrying out the present studies.

Location:

Dasuya is a small town in Hoshiarpur district and is situated at a distance of about 25 miles from Hoshiarpur in the north west on the Hoshiarpur-Dukerian road and is...
tehsil headquarters. It is situated at a point 30° 46' North, 75° 32' East. The district of Hoshiarpur is bounded on the east by the districts of Kangra, Simla and Bilaspur; on the west by Jullundur and Kapurthala districts; on the north west across the river Beas by Gurdaspur district and on the south across the river Sutlej by Ambala district.

Out of the six villages selected for studying population dynamics of Oxva in Dasuya area, three are situated on the west side of the Jullundur—Pathankot motor road, while the remaining three are on the eastern side of this road. The distance of these six stations, viz., Mangat, Usman Shaheed, Saddarpur, Chack Quasam, Uchi Bassi and Fatehgarh from Dasuya towns is 3, 1, 3½, 2, 3 and 4 4½ miles respectively. The distances of these stations from the river Beas, which is running along the north western side of the tract, are Mangat 0.75; Usman Shaheed, 2.5; Saddarpur 3.75; Chack Quasam 3.25, Uchi Bassi 4.5 and Fatehgarh 7 miles.

**Climate:**

Dasuya area falls in the submountaneous region. The annual rainfall is about 968.9 mm. (Appendix I). From the point of view of the rainfall, the year can be broken into 4 periods, viz., winter (October—February), spring (March—April), summer (May—June) and the rainy season (July—September). The winter months usually receive just a little rain, i.e., 206.4 mm. in five months. The climate during
this period, specially in December-January, is very cold, the average temperature being 15.57°C; the maximum temperature 35.0°C; and the minimum temperature 0°C. These months occasionally experience frost and hail storms.

The spring months (March-April) receive very little rainfall, a total of 85.57 mm. or so and have a mild climate, the average temperature of 20.23°C; the maximum of 37.0°C and the minimum of 8.0°C. The summer months (May-June) are very dry and hot with a rainfall of 76.80 mm.; the average temperature of 31.77°C; the maximum of 45.5°C; and the minimum of 11.0°C and dust storms are the common feature of this period. It is during the rainy months of July-September that the most of the rain falls, i.e., 598.1 mm. on average. The climate of the area is hot and damp, the average temperature being 29.24; the maximum 39°C; and the minimum 21°C.

Nature of the soils under study:

1. The soil of village Mangat under study was loamy in texture, alkaline in reaction, with pH 7.9 and contained low amounts of soluble salts with a conductivity of less than 0.10. The amount of organic matter and available phosphorus was also low.

2. The soil of village Usman Shaheed was also loamy in texture, with a slightly alkaline reaction(pH 7.6) and a conductivity of 0.75 millimeters per cu. The proportion of organic matter and available phosphorus are low here, too. Check Qusam.

3. Saddarpur, Uchi Bassi and Fatehgarh soils were loamy sand in texture and slightly acidic in reaction, with a low conductivity. The presence of organic carbon
and phosphorus was also low.

Cropping pattern:

The major crops of the area during the kharif season (April-September) comprised rice, maize, sugarcane and kharif fodders (chari, guara and beans), whereas during rabi season (October-March) wheat, wheat and gram mixture and rabi fodders (berseem, shaftal and senji) were mainly grown. The area under different crops for each station, for the years 1941-1961, is presented in Appendix II.

The rice formed an important crop of the kharif season in the wet region of Mangat, Usman Shaheed and Saddarpur and the area under this crop increased every year. In the last two decades there was no increase in sugarcane area and maize crop had a severe set back, particularly in Mangat and Usman Shaheed. Maize and sugarcane were grown in the dry region in small areas and most of the land remained fallow during the kharif season. All the major kharif crops in the tract were subjected to heavy losses by Oxya.

Appendix II further shows that berseem, which has become the main fodder of the rabi season, was grown in Mangat, Usman Shaheed and Saddarpur, whereas, on the eastern side of the road, the area under this fodder was very little. The cultivation of berseem in this tract started in the early fifties and after that there has been a steep rise in its popularity. And this was the only cultivated crop of rabi season which was attacked by this pest.
The natural vegetation of the area represented a characteristic feature which divided the whole tract into two regions, viz., the wet and the dry. The important grasses and weeds of this tract included swank (*Panicum colorum* Linn.), beri swank (*Echinochloa colorum* Linn.), motha (*Cyperus rotundus* Linn.), ohiri ka dana (*Setaria glans* Linn.), madhna grass (*Dactyloctenium aegyptium* Linn.), water plant or Polygonum (*Polygonum coccineum*), horse tail (*Equisetum* spp.), *Tynha angustata* Bory and Chaub., *T.elephantina* Roxb., and tippy grass. The vegetation of the dry area, however, comprised of fewer varieties. The important are *kuhsa* grass (*Desmostachya bipinnata*), *anjem* grass (*Cenchrus ciliaris* Linn.), *takri* grass (*Digitaria adscendens* H.Hk) Henr. and *Jhau*.

Bary (*Sorghum halepense* Linn.), kahi (*Saccharum spontaneum* Linn.), sirkanda (*S.munja* Roxb.) and khabal grass (*Cynodon dactylon*) were present in both the dry and the wet regions. The stand of the vegetation in the wet region was very dense and almost all the fallow fields remained covered with one of the other weed.

However, their stand was recorded on light soil.

Out of all the weeds and grasses, *Panicum colorum* and *Cynodon dactylon* were the most favourite food of *Oxya*.

Saccharum munja, though not liked much as food, was preferred for oviposition by the insect.

In Dasuya area six villages, representing different types of conditions, i.e., both wet and dry, were selected. From each village, a compact block of about one hundred acres was selected for the ecological studies.
observations regarding the population dynamics of *Oxya* were recorded from each block and the meteorological observations were recorded daily.

ii. **Collection of meteorological data:**

Maximum-minimum and dry wet bulb thermometers were installed at each station in the meteorological screen (15 cm x 16 cm x 15 cm). The soil temperature at a depth of 2 cm was recorded with an ordinary thermometer, by fixing the thermometer two cm below the soil surface and the degree of it was recorded when the temperature became constant. The relative humidity was recorded with the help of a whirling psychrometer. These meteorological observations were recorded daily between 1½ to 2 hours after sun-rise. Efforts were made to record the observations at that particular time daily from each plot.

iii. **Method of recording *Oxya* population:**

At each station ten different plots, representing five representative vegetations, were selected for recording *Oxya* population. Five sweeps with hand-net at a time, each from ten different plots, already selected, were taken at an interval of one week. To minimise the error, efforts were made to record the population at the same time every week.

**Methods of Recording Population:**

Different methods of recording the population of locusts and grasshoppers have been evolved by different workers to suit their requirements. These methods were tried in assessing the population of *Oxya* and are discussed below:-
1. Counting while walking:— The insects which were seen by the observer while walking at a standard pace, were counted. It was observed that the density of population of *Oxya* was too high for being conveniently assessed by this method. Lean (1936), Smith (1939) and Rao (1942) also have remarked that the method of counting and recording acridid population while walking, should only be practised when the density of population is low. Moreover, Joyce (1952), has called this method unreliable.

2. Quadrat method:— This is the principal method employed for estimating the number of hoppers and adults in the field and the population of the insect is counted in a number of quadrats distributed over the entire infested area, (Uvarov et al, 1951; Richard and Waloff, 1954; Dempster, 1957). This method could only be practised if the population was not affected by immigration or emigration i.e. the infested area should be bounded by such barriers as roads, rivers or hills (Personal communication dated 10.9.1959 to the writer from Dr. O.W. Richard). But there were no such barriers bounding the insect-inhabited area in Dasuya. Therefore, the quadrat method was not adopted in assessing the *Oxya* population there.

3. Portable enclosure method:— Portable enclosures of different sizes have often been used for estimating the population of grasshoppers and locusts and this method has been considered to be the most reliable method, too. This method was, accordingly, used in the present case.
Portable enclosures for recording the density of population were used by Rubtsov (1935); Konakov and Onisimova (1936); Vinokurov (1938); Prebble (1943) and Singh & Rhatia (1952).

A wooden frame, with galvanized sheet edges, enclosing an area of one yard square, was held suspended by means of two long ropes horizontally fixed to its two parallel sides and was quickly placed over the infested area from a distance when the insects were inactive. This method was employed in fields having such vegetation as khambal, swank, dila, maize, sugarcane and rice, ranging from 6" to 40" in height. It was observed that the frame did not succeed properly when the height of the crop was above 20" and the stem of the plant was stout enough not to bend or to break under the weight of the frame. Moreover, hard stems under the frame made the escape of the insects very easy, as the stems did not permit the frame to touch the soil. Thus, this method gave reliable results in areas that were covered with khambal (6") and maize (18") plus swank and dila (12" each). The frame was covered all over with a muslin cloth. After landing the frame on the area, the insects were searched, caught, counted and released there and then. The data are presented in Table 1.
Table 1

Data relating to the catch of grasshoppers with a portable frame.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Maize+swank</th>
<th>khabbal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Height</td>
<td>12&quot;-18&quot;</td>
<td>6&quot;</td>
</tr>
<tr>
<td>2. No.of observations.</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>3. No.of Oxya.</td>
<td>257</td>
<td>129</td>
</tr>
<tr>
<td>4. Area covered.</td>
<td>450 sq.ft.</td>
<td>450 sq.ft.</td>
</tr>
<tr>
<td>5. Calculated population per acre.</td>
<td>24878</td>
<td>12487</td>
</tr>
</tbody>
</table>

Under cropped conditions this method of estimating the population of grasshoppers was not found to be practicable, as the use of frame damaged the crops. The damaged plants in later stages were not able to recoup and the accumulated damage over a number of times resulted in considerable loss in yield.

4. Method of marking and recapture:– This sampling method was widely used in studying animal population (Dempster, 1957). In this case the population must be confining to occupy a definite area with no inward or outward movement. But in the case of Oxya, there was no check on the movement of the insects and the counting of the population was thus rather uncertain. Moreover, young hoppers were too fragile for marking and moulting in the nymphal stage also reduced the efficacy of this method to be practised for this insect.
5. **Sweeping with hand-net method:** The use of sweeping with a hand-net recommended to estimate the insect population has been mentioned as the cheapest and simplest method. This method had either been used for determining the density of the population (Hoerner and Longford, 1925; Rubtsov, 1932; Joyce, 1952; Flock and Deal, 1959) or adopted for recording the composition of the population of these insects (Wilber and Fritz, 1940; Dobzhansky and Parran, 1950; Richard and Waloff, 1954; Dempster, 1957). It was not possible to determine the actual number of insects present in a specific population with this technique.

Out of the four methods of estimating the acridid population sweeping with hand-net method would seem to be the best for estimating the population of *Oxva nitidula* under the present circumstances. This method was, therefore, used throughout the year, for estimating the population of all the post-embryonic stages of the insect on all types of vegetations in this case. At each station ten different plots, having five selected vegetations in them, were chosen for recording the population of *Oxva*. From each of the ten plots, five sweeps with hand-net were taken at an interval of one week. The insects thus trapped were identified, counted and released at the site.

**Statistical methods:**

Statistical methods of correlation, simple and multiple regression and analysis of variance were used for the analysis of the data.