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Some Effects of a Constant Temperature and Different Relative Humidities Upon Egg Laying of Melanoplus Bivittatus Scudder.

Millard Jewell Hatten

SOME EFFECTS OF A CONSTANT TEMPERATURE AND DIFFERENT RELATIVE HUMIDITIES UPON EGG LAYING OF MELANOPLUS BIVITTATUS SCUDDER.

By

Millard Jewell Hatten

A thesis submitted to the faculty of the South Dakota State College of Agriculture and Mechanic Arts in partial fulfillment of the requirements for the degree of Master of Science.

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To Professor H. C. Severin, I wish to extend my thanks for suggesting that the problem be investigated and for the many helpful suggestions and criticisms offered throughout the investigation.

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Helpful suggestions were received from Miss Nelle Hartwig, who on several occasions assisted materially in the preparation of some of the equipment. Mr. Paul Jones, a student, I wish to thank for the diligent care of the cages used in the outdoor experiments for one month.

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SOME EFFECTS OF A CONSTANT TEMPERATURE AND DIFFERENT RELATIVE HUMIDITIES UPON EGG LAYING OF MELANOPLUS BIVITTATUS SCUDDER

INTRODUCTION

Of the various species of grasshoppers occurring in rather extensive numbers year after year in the northwestern plains states, Melanoplus bivittatus is one of the most prevalent and destructive. It was thought that a study of the influence of different temperatures and relative humidities upon the reproductive potential of this species might reveal some information and that this information might be of some value in an attempt to forecast the abundance of this grasshopper in years following others in which the factors of temperature and relative humidity were known.

A search through all the available literature failed to reveal any specific information pertaining to this particular phase of ecological work upon Melanoplus bivittatus. However, several publications dealing with other species of grasshoppers were encountered. The one considered to be of the greatest help in making an approach to the present studies was the publication prepared by Dr. J. R. Parker (1). Mr. Parker, however, carried on his experimental work with Melanoplus mexicanus mexicanus Sauss., Melanoplus femur-rubrum femur-rubrum (De Geer) and Camnula pellucida Scudder. The work of Mr. Parker is referred to on several occasions in this thesis and greatly influenced the writer in drawing certain conclusions.

The benefits derived by the writer from the present studies can not be measured by the information revealed in the form of scientific data in this publication, for it must be taken into consideration that this was his first attempt to carry on a project of this nature, and the available equipment as well as the time were limited during the entire period the problem was under investigation. However, good experience was obtained and some observations were made that have proven invaluable to the writer. It is hoped that through this thesis some information of value will be passed on to those interested inecological work.

The work on these studies was conducted in the Entomology-Zoology Department of South Dakota State College. The period over which the investigation was carried on extended from October, 1930 to March, 1932, inclusively. The information from sources other than that derived from the investigations and used in formulating this manuscript will be given due credit in their proper place in this manuscript. Included in the thesis will be found a bibliography of the most important references consulted during the period the problem was under investigation.

SCOPE OF THE INVESTIGATION

When the project for investigation was first assigned the writer, the scope of the problem included a study of the effects of temperature and humidity upon the egg laying of Dissosteira carolina (L.), in addition to that of Melanoplus bivittatus (Say). Because of the scarcity of the carolina species in the fall when the investigation was started and because the few specimens that were caught seemed to be of a delicate nature and died after having been confined in cages only a few days, it was thought best to postpone work on this species until the following summer.

The following summer another attempt was instituted to include this species in our investigations, and consequently a number of specimens were caught and caged for a few days. The mortality of these specimens proved to be so great that finally it was decided to drop a study of this species entirely.

After the investigation under cabinet conditions had been under way for some time, it was decided that a study of the reproductive potential of Melanoplus bivittatus under outdoor conditions would prove worth while. This particular work was started in the summer of 1931.

No attempt was made during this entire investigation to systematically study the morphology or life history of Melanoplus bivittatus.

METHODS OF CONTROLLING TEMPERATURE

A constant temperature could be maintained in our cabinet with much less difficulty than a constant relative humidity. As has been already stated, the heat used in raising the temperature of the cabinet to the desired degree was obtained through a battery of Mazda lamps kept under thermostatic control. Because the cabinet was kept in the south laboratory of the building in which the Entomology-Zoology Department is housed, and since this laboratory was fairly warm at all times, it was necessary to use only one 75-watt lamp in our heating unit to raise the temperature of the cabinet to the desired degree.

A temperature of 27° C. was the only constant temperature used in this investigation, due to the fact that more time was required to find a means of controlling the humidity than was thought would be necessary in the beginning. The use of a temperature of 27° C. was chosen in preference to other temperatures due to the fact that Parker (1) found this temperature to be the one at which the highest acceleration of egg development took place with the species of grasshopper studied by him, provided humidity conditions were favorable.

Lower temperatures were not used by us due to the fact that equipment as well as time prohibited this. To use lower temperatures would have necessitated the installation of some form of refrigeration, and for such installation, funds were not available. It had been the intention of the writer to use a lower temperature than 27° C. by placing the cabinet in the basement of the greenhouse, but time did not permit.

Criticisms:

There were only a few difficulties encountered in our attempts to obtain and maintain a constant temperature in our cabinet. Throughout our work there was seldom a larger variation in temperature than a single degree F. Occasionally, however, a greater variation was experienced, the cause of the fluctuation being found in the fusing of the points on the thermostat. This fault was readily corrected by sand papering the points. By keeping the surfaces of the points smooth at all times, this difficulty was eliminated.

Conclusions:

It has been the writer's experience that temperature can be readily and accurately controlled in a constant temperature cabinet. The only serious difficulty that was experienced was due to a fusing of the points of the thermostat, and this may readily be avoided by keeping the points smooth by sand papering them.

METHODS OF LIGHTING CABINET

Light was supplied to the cabinet through a 75-watt Mazda lamp, which was placed over a window in the top of the cabinet. This window measured 6 inches square. Ordinary double strength window glass was used in the construction of the window, but instead of a single pane, two panes of glass were used, the two being separated by an air space of about an inch.

The Mazda lamp was hung about an inch directly above the window in the cabinet. Further, the lamp was provided with a good reflector, which reflected the light into the cabinet. The insects used in the investigation were placed in containers that were kept in the cabinet immediately beneath the window.

The lamps that supplied the heat to the cabinet were ordinary Mazda lamps. They were not painted and were under thermostatic control.

No attempt was made in this investigation to study the effect of light upon the reproductive potential of Melanoplus bivittatus. However, it would not be difficult to carry on such an experiment. The literature consulted gave us no information along this line so far as grasshoppers are concerned, although data are available on the influence of light upon egg-production in other insects.

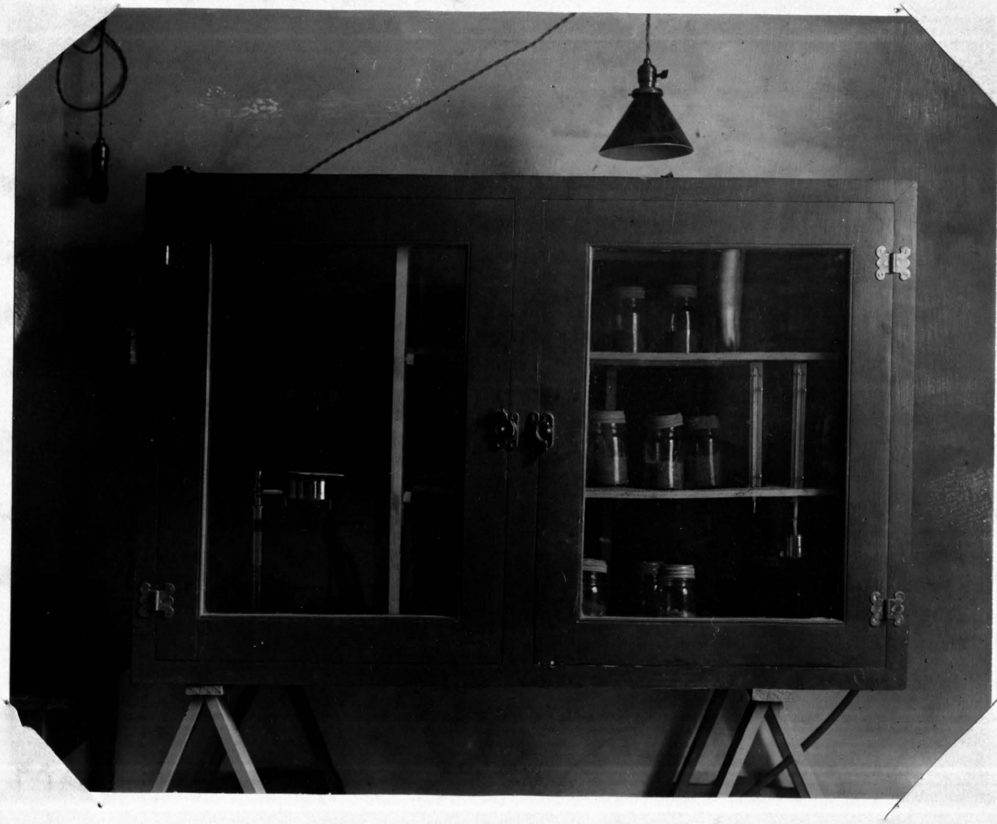

PLATE I. - Photograph showing cabinet and arrangement of apparatus used in controlling temperature and relative humidities during the investigation.

The cabinet used by the writer, though not absolutely air-tight, required only a small amount of additional heat occasionally to maintain the same constant temperature under ordinary conditions. Through the temperature mechanism control apparatus that we used, the variation of temperature, excepting in a few cases, was less than one degree. Rarely did the temperature vary more than one degree, and whenever this happened, it was due to fusing of the points of the thermostat. This will be discussed later.

The cabinet was equipped with the most recently used electrical devices for regulating temperatures and relative humidities. These devices will be discussed more in detail in another section of this thesis.

METHODS OF PROCEDURE USED IN CABINET INVESTIGATION

When the cabinet experiments were begun it was planned to study the effect of different constant degrees of temperature and relative humidities upon the reproductive potential of Melanoplus bivittatus.

While attempting to find a suitable method by which the factors of temperature and relative humidity could be standardized and maintained at a reasonable degree of accuracy, certain difficulties were encountered and ultimately it was possible to use only one constant temperature during the investigation.

However, a method was perfected whereby humidity could be controlled fairly accurately at all times, with the consequence that several different degrees of relative humidity were used. As a result, more data and information were made possible on the effects of humidity upon egg laying than on the effects of temperatures, and as a consequence the thesis will be found to deal more with the former factor.

SOURCE OF MATERIAL

It was thought advisable to begin the experimental work by collecting a number of adult Melanoplus bivittatus. With this idea in mind, twenty pairs of adults were collected from nearby fields early in October and placed in tall, cylindrical glass jars which had been filled to a depth of four inches with sand that was sifted through a twelve-mesh screen (Plate II). The tops of these jars were covered with a single thickness of cheese cloth held tightly in place by one-eighth inch rubber bands.

The jars containing the mature grasshoppers were placed in the greenhouse, and as a result the egg-laying period of the grasshoppers, which had no doubt already been underway for some time, was prolonged. The temperature in the greenhouse, though not constant, was high enough to keep the grasshoppers active at all times, and probably ranged from as low as 60° F. to as high as 90° F.

The insects were fed daily, and the sand was kept in a moist condition at all times until the grasshoppers died. No attempt was made to keep any daily record of the length of life of the individuals, but from observations and notes taken irregularly, it was found that the first death occurred in ten days, while the last female lived until the fifteenth day of November.

Attempts to hatch the eggs laid in these containers and attempts to rear these young hoppers to maturity will be dis-

PLATE II.- Photograph of the battery jars used in confining grasshoppers while in the greenhouse.

cussed under rearing methods, but it might be said at this time that these attempts were rather disappointing, and as a consequence the progress of the thesis was delayed considerably.

After having encountered the above-mentioned difficulties, it was decided to adopt another procedure of securing adult two-striped grasshoppers that had not yet begun egg-laying. The method adopted was suggested by Professor Severin and will now be described.

Close watch was kept of a colony of two-striped grasshoppers living in the open under natural conditions. As soon as members of the colony became fully winged, such specimens were collected and later used for experimental purposes. In order that there might be no mistake made, 100 of such females were caught, dissected and the ovaries examined. Only two of these females showed well developed eggs in the ovaries, while in the remainder no well developed eggs were found. Further, the abdomen of the two specimens mentioned was long and firm, while in all of the 98 remaining females, the abdomen was comparatively shorter and softer.

By following this procedure, it was possible to work with male and female Melanoplus bivittatus that acquired their development in a normal environment. Such specimens were hardy and normal so far as size and morphology are concerned.

REARING METHODS

During the period the problem was under observation, several methods were employed in attempting to find a suitable way by which the newly hatched young grasshoppers could be reared to mature insects.

In rearing grasshoppers, Parker (1930) found glass tubing a satisfactory container in which to rear the young to maturity. Carruth (1930), in studying the life history of the Croton bug, used similar tubing, and was satisfied with the results obtained. The success experienced by these workers in their rearing work prompted the writer to use similar containers for rearing the young grasshoppers to adults in this investigation.

The first type of rearing tubes used by the writer were made from test tubes eight inches long and one inch in diameter. Each test tube was cut into two parts, thus making two tubes, each one inch in diameter and four inches long. One end of each tube was closed with a cork, which had a $\frac{1}{4}$-inch hole bored through its center, the hole being covered with copper gauze held in place by small tacks. The opposite end of the tube was closed by one thickness of cheese cloth held in place by rubber bands. This type of container allowed the entrance of all available light and provided circulation of air through the tubing.

The rearing tubes described were identical to those used by Carruth (1930), except for the following difference.

Carruth dipped the corked ends of his tubes in hot paraffin and following this, fastened the copper gauze over the hole of each cork with tacks. It was the experience of the writer that as such tubes were used, the continuous coating of paraffin sealing the corks in the tubes sometimes became broken and since the corks, because of their coating of paraffin, were slippery, the corks had a tendency to work out of the glass tubes. It was necessary to dip such tubes in hot paraffin as often as the corks would work loose. To avoid the objections discussed, it was decided to omit the paraffin treatment of the rearing tubes, and this in large measure remedied the objectionable feature.

After the tubes described were used for a time, it was found that they were not satisfactory, possibly because of their small size. Consequently two other sizes of tubing were used in an attempt to find a more suitable type of container in which to confine the young grasshoppers, the first size measuring 1¼ inches in diameter and 8 inches in length. The second tubing used was 3 inches in diameter and 16 inches in length.

In general, it may be said that none of the rearing tubes were entirely satisfactory, for none could be made to simulate very closely the natural environment of the grasshoppers. However, the smallest tubes were less satisfactory than the intermediate and largest sizes. There was little difference, if any, in the results of rearing the hoppers in the intermediate and largest tubes.

Food given these insects in the winter consisted of lettuce leaves which were taken from lettuce heads obtained from local grocery stores. In the spring and summer, dandelion leaves (Taraxacum) and alfalfa stems, leaves and flowers were supplied instead. Moisture was added to the tubes by placing in each of them a piece of white blotting paper, 1 inch wide and 6 inches long, and this was moistened with water daily by means of a dropper.

Criticisms:

In using the tubes described as a means of rearing insects, we have containers which allow all the available light possible to reach the confined insects. A fair movement of air through the tube is also possible, but then we have conditions to deal with which in most cases prove rather unsatisfactory.

The main objection to the use of cylindrical glass tubing as containers in which to rear grasshoppers is that the tubing does not allow enough space for the natural movement of the insects. Locomotion of grasshoppers is by three means--first, by the use of all pairs of legs, called walking; second, a method called jumping, in which the third pair of legs are chiefly used; and third, flying.

In confining grasshoppers in tubing we deprive them partially in some respects, and completely in others, of the opportunity of performing their normal locomotory movements. It is possible, however, that this may not affect them materially, but then again we know that their environment has been somewhat modified and there is a possibility that they are not able to adjust themselves to this more sedentary life.

Even though the confined method of living may not directly effect the grasshoppers in an injurious way, the attempt of the grasshoppers to use their legs as if they lived out-of-doors and unconfined is not decreased. When one approaches the tubes containing these insects, the grasshoppers begin to jump, bumping against the tubing again and again which, though it may not be harmful if done only a few times, when repeated for several weeks surely does not help them.

In our rearing work in which we employed the method heretofore discussed, only one specimen out of 50 reached the adult stage in normal size and with all its appendages intact. The loss of the appendages in most of our specimens no doubt was due in part to the absence in the rearing tubes of objects on which the molting insects might climb. Undoubtedly large containers with objects upon which the grasshoppers could climb when molting would greatly reduce these losses.

Light probably effects the normal development of grasshoppers, but how this is done we have no way of knowing. The effect of its absence is likewise not known.

Conclusions:

The experience of using glass tubing of various sizes in which insects were reared proved to me that the larger the tube the better the results. Of course it must be taken into consideration that in investigations in which individual insects are to be observed separately, that it is almost impossible as well as impracticable to use large containers. This procedure, if followed, would involve more expense, labor and room, and the results might not be so different after all, especially if the larger containers were kept in cabinets.

If the rearing of immature to mature insects was the object in mind, it would be much more satisfactory if the insects were grouped in larger containers, thereby eliminating considerable labor, equipment and expense. In this way better movement of air could be obtained through the rearing containers and more space provided.

METHODS OF CONTROLLING HUMIDITY

Means by which a constant relative humidity could be maintained at all times during the period the insects were held in the cabinet constituted the most difficult problem of the cabinet work. Several methods were tried to regulate the relative humidity, but none were entirely satisfactory.

In the first two methods, the insects were placed in pint Mason fruit jars, but the top of each cover of these jars had been cut out and a piece of copper screen of 16 mesh to the inch had been carefully soldered in its place. In the third method used to regulate the relative humidity, the grasshoppers were again confined in Mason fruit jars, but because of a radical change in the equipment used in regulating the relative humidity, the containers were changed materially. This change in construction of the containers will be described later.

The principle used in maintaining a constant relative humidity in our first series of experiments was briefly as follows: A container of a suitable size and holding water was placed in the cabinet and then an electric fan was used to circulate a current of air over the surface of the water. When the desired relative humidity was reached, an automatic cut off stopped the rotation of the fan.

With this plan in mind a galvanized tin box, 6 inches wide, 6 inches high and 12 inches long was constructed. The box was fitted with a cover lined with felt. When the cover was fitted over the box, evaporation of the water was largely prevented when the fan was not operating, and by raising the cover, water could be added to the box when necessary.

In one end of this box was mounted a small electric motor which had previously been used for operating a six-inch fan. The fan, because it was too large for the box, was replaced by one made by hand from material similar to that used in the construction of the box. The fan measured 3 inches in diameter and the blades were given a curvature possessed by the original fan. In the bottom and center of this box was soldered a one-inch strip of tin, extending entirely across the box and dividing the lower half of the box into two compartments. The purpose of this division was to prevent the water placed in one compartment from coming in contact with the leaves of the fan in the other compartment. Directly above this strip at the bottom and just beneath the cover, a one-fourth-inch strip of tin was soldered at each end to the sides of the box to give rigidity to the box and at the same time to support six other similar strips perpendicular to it and running to the end away from the motor. These strips, running lengthwise of the box, were used as supports for holding blotting paper, which dipped into the water below.

At the end of the box containing the motor, two one-half-inch holes were bored. These permitted air to be drawn into the box. The air was then blown over the blotting paper and out of the box at its opposite end thru a series of holes one-eighth inch in diameter.

The frequency with which the motor was put in motion was controlled by a hygrostat. The type of hygrostat used in this experiment worked on the principle of the hygroscopic coefficient of human hair. This hygrostat was constructed in the laboratory, the framework consisting of heavy galvanized tin. It was thought that by using metal instead of wood, an objectionable feature of expansion or shrinkage of wood would be greatly reduced. The group of hair, containing the hygrostat proper, consisted of about 16 strands and was fastened to one end of the framework of the hygrostat by a set screw, which made possible adjustments as long as the one-fourth-inch screw that was used. The other end of the strand of hair was fastened to a spring located at the other end of the framework.

The hygrostat was connected to the electric current of the building. The high current was reduced by means of a telegraphic relay controlled by three dry cell batteries. The points on the relay were small and composed of a soft metal, and therefore had a tendency to fuse. This objectionable feature was eliminated by placing carbon points over the metal, thus giving the points a greater exposure of surface and a harder material.

Whenever the humidity was lowered beyond a certain point, the strand of hair contracted. The result was that the points on the relay came together, and the motor was put in action. When the hair became damp it lengthened and the contact was broken. This type of hygrostat is commonly used in work of this nature. However, the accuracy of such a hygrostat will be discussed later.

The second method used in regulating the relative humidity was primarily the same as the first, the only difference being that the fan was now placed in the center of the humidity box and a series of rows of blotting paper was used at each end (Plate III). In this way the air was passed over twice the surface of wet blotting paper as before. In addition, the box was slightly increased in size.

These methods, though involving the same principles, were found to give very different results. Thru the first method, a four-hour period was found necessary to build up a relative humidity of 70°, while thru the latter, only a few minutes were necessary.

The change in apparatus increased the surface evaporation of the water to such an extent that condensation of moisture took place in the cabinet in spite of the fact that a six-inch fan was kept continually in circulation. It was thought that by setting the hygrostat to a lower humidity, this fault could be eliminated, but this was done with no success, and finally the method was entirely dropped as a means of obtaining a constant relative humidity.

The method next attempted and which was the one finally used was a modification of Headlee's method of obtaining constant relative humidities by drawing air through saturated solutions of chemicals. By arranging the jars containing the different solutions and insects in a series, running from the lowest relative humidity to the highest, one stream of air could be used for the entire series.

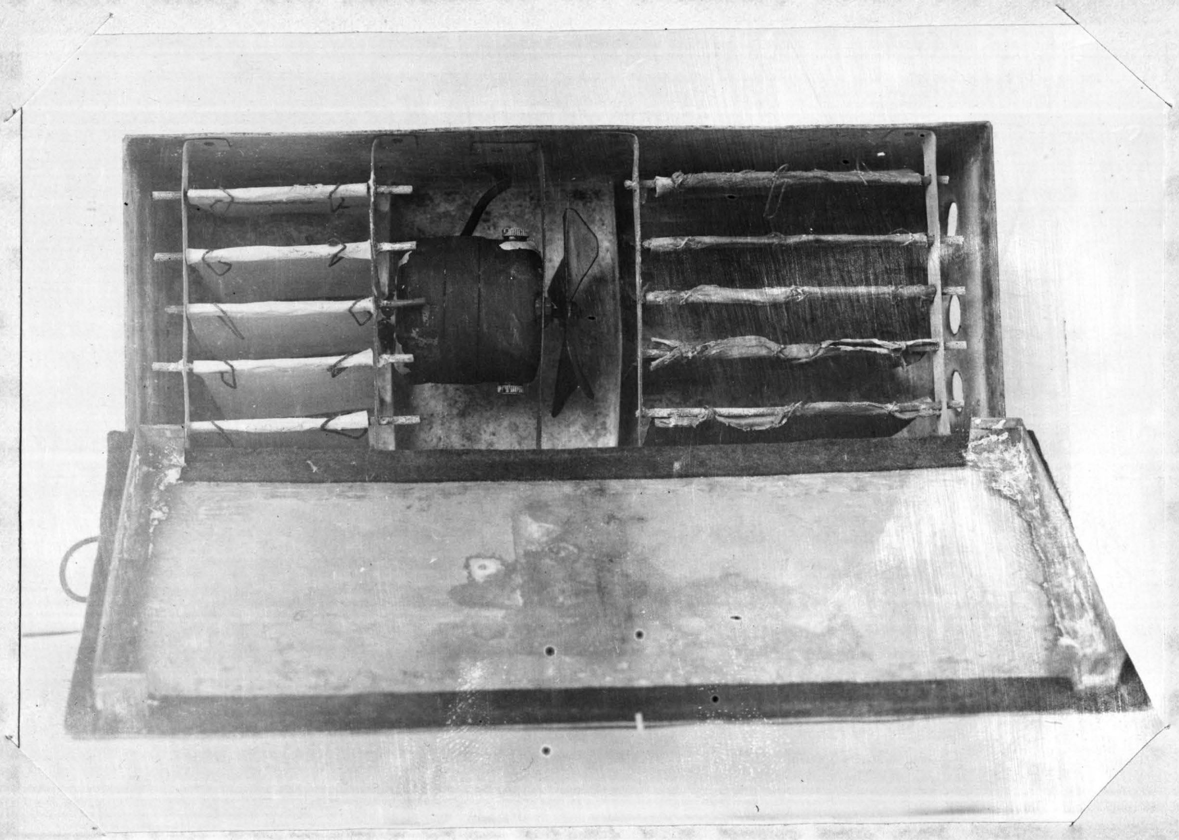

PLATE III - Top view of humidifier with cover removed. This humidifier was used in obtaining the relative humidities used in our cabinet experiments.

This necessitated the supplanting of the open top type of jar with a container which could be made air-tight. Again fruit jars were used, but instead of the ordinary screw top type, the easy sealing type of jars was used. By supplanting the glass covers with tops made of wood, holes could be bored in these tops. Through these holes, glass tubing could be inserted and by means of rubber tubing the jars containing the different solutions and the jars containing the insects could be connected together in a series. A filter pump which was controlled by a hygrostat similar in construction to that already described was used to pull the air current through the series of jars already mentioned.

The hygrostat with a precision hair hygrometer was placed in a sealed jar within the cabinet. This jar was placed between the container inclosing the solution of the highest concentration of chemical used and the first jar of insects. The hygrostat regulated the frequency with which the pump was put into operation and the hygrometer gave a check on the stability of the solution of the highest concentration of the chemical used.

The chemical solutions used to obtain the constant relative humidities employed in this investigation were gotten by combining sulphuric acid and distilled water. The following table taken from Parker's work (Table I, p. 10) shows the humidities obtainable by using different concentrations of sulphuric acid.

Per cent of sulphuric acid by weight	Specific gravity of solution	Resulting humidity
67.4	1.58	10 per cent
58.7	1.49	20 " "
53.1	1.43	30 " "
48.1	1.385	40 " "
43.5	1.335	50 " "
39.9	1.300	60 " "
34.0	1.255	70 " "
27.0	1.195	80 " "
17.0	1.135	90 " "
0	1.000	100 " "

Criticisms:

Relative humidity is the most difficult environmental factor to control in a cabinet. Claims made by some workers that the apparatus used by them, and which was very similar to that used in this work, controls the relative humidity with less than one-tenth of a degree of inaccuracy are misleading. Many factors are likely to cause error, two of which are dust and acid fumes.

Hair that is to be used in making a hygrometer should be washed in ether in order to dissolve any fat or oil that may occur on or in the hair. If this is not done, or if the hair is handled carelessly, the hair again becomes contaminated, thus reducing its efficiency.

Dust, when it lodges on the hair, also reduces the efficiency of the hygrometer. This is probably done largely by reducing the hair surface that is exposed. Undoubtedly an exposure of the hair to acid fumes effects the hair materially, for in checking hygrometers exposed varying length to acid fumes, they were found to vary materially.

Conclusions:

Because of the inaccuracy of most of the methods used in controlling humidity and because of the expense entailed in the use of the more accurate methods, a field presents itself in which someone mechanically inclined could do some valuable investigational work. There is an urgent need for an accurate and practical apparatus to control relative humidity, and in addition such an apparatus should be moderately priced.

METHODS OF CONTROLLING TEMPERATURE

A constant temperature could be maintained in our cabinet with much less difficulty than a constant relative humidity. As has been already stated, the heat used in raising the temperature of the cabinet to the desired degree was obtained through a battery of Mazda lamps kept under thermostatic control. Because the cabinet was kept in the south laboratory of the building in which the Entomology-Zoology Department is housed, and since this laboratory was fairly warm at all times, it was necessary to use only one 75-watt lamp in our heating unit to raise the temperature of the cabinet to the desired degree.

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Light was supplied to the cabinet through a 75-watt Mazda lamp, which was placed over a window in the top of the cabinet. This window measured 6 inches square. Ordinary double strength window glass was used in the construction of the window, but instead of a single pane, two panes of glass were used, the two being separated by an air space of about an inch.

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No attempt was made in this investigation to study the effect of light upon the reproductive potential of Melanoplus bivittatus. However, it would not be difficult to carry on such an experiment. The literature consulted gave us no information along this line so far as grasshoppers are concerned, although data are available on the influence of light upon egg-production in other insects.

TYPE OF CONTAINERS IN WHICH ADULT GRASSHOPPERS WERE CONFINED IN THE CABINET DURING THE INVESTIGATION.

The containers in which adult grasshoppers were confined and then placed in the cabinet for investigation, were described in general under Methods of Controlling Humidity. When the Mason fruit jars were used, the top of each cover was cut out and in its place a piece of copper screen of 16 meshes to the inch was soldered. The tops of such jars could be conveniently screwed off and on as often as necessary, thus making it an easy task to add fresh food to the jar, to remove old food, excreta, etc. However, such jars proved unsatisfactory, for the same exact relative humidity could not be maintained in them.

The second type of container that was used was the easy sealing fruit jar. The glass covers were discarded and wooden plugs were substituted for them. To make the plugs fit as air-tight as possible, their edges were smeared with vaseline. Two holes were bored in each plug, smeared with vaseline and through the holes glass tubing was inserted, in order that air of a constant relative humidity might be sucked through the jars. However, it was very difficult to keep the wooden plugs absolutely air tight, regardless of the amount of care that was exercised. The development of some container that can be easily manipulated and that would be satisfactory in experimental work of this sort is highly desirable.

INFLUENCE OF TEMPERATURE UPON EGG LAYING

Because in our experiments, the grasshoppers were subjected to only one constant temperature (27° C.) and because of lack of time to repeat such experiments under different temperatures, it is impossible to state definitely that egg laying in Melanoplus bivittatus is effected materially through different constant temperatures.

However, Parker after studying the effect of different constant temperatures upon Melanoplus mexicanus and Camnula pellucida concluded that egg laying is definitely influenced in these species through temperature. With the former species he found that egg laying was never observed to occur at an air temperature lower than 69° F. From 12 observations made on this species, he concluded that egg laying began on an average at 71.7° F. air temperature or 99.8° F. soil surface temperature. With the second species a somewhat higher temperature was required, namely 72.4° F. air temperature and 101.3° F. soil surface temperature. With both species, egg laying continued as the temperature rose, but at about 80° F. air temperature (107° to 113° F. soil surface temperature) egg laying ceased, and the grasshoppers were forced to climb vegetation to escape the heat of the soil. However, egg laying may continue at the higher temperatures because some of the grasshoppers locate shady and, therefore, cooler spots of soil.

From Parker's data, one would naturally predict that egg-laying in Melanoplus bivittatus is also influenced through temperature. Further studies with this species of grasshopper and with varying temperatures as ecological factors should decide this matter.

INFLUENCE OF MOISTURE UPON EGG LAYING

Studying the influence of moisture upon egg laying of adult grasshoppers is a much more difficult problem than studying the effects of temperatures. To maintain a constant relative humidity at the desired percentage proved to be a problem which was never satisfactorily solved. So far as known, only a carrier air conditioning cabinet would have produced suitable conditions, and the cost of this type of humidifier was in excess of available funds.

Six different degrees of relative humidity were used in our experiments, and with each humidity, the experiment was repeated three times. The different humidities used were 40%, 50%, 60%, 70%, 80%, and 100%. A constant temperature of 27° C. was maintained throughout the duration of all of the experiments.

Due to the fact that the numbers of grasshoppers experimented upon were so small, it is impossible to draw any definite conclusions from the experimental data obtained. The data obtained is to be found in table II of this thesis.

TABLE II. - Influence of different relative humidities upon the reproduction potential of Melanoplus bivittatus.

Per cent relative humidity	Jar number	Number of egg pods laid by each female	Average number of egg pods laid by each female grasshopper
40%	1 2 3	1 4 3	2 2/3
50%	1 2 3	1 4 1	2
60%	1 2 3	2 3 1	2
70%	1 2 3	1 2 2	1 2/3
80%	1 2 3	3 1 1	1 2/3
100%	1 2 3	2 0 2	1 1/3
Total		34	

THE LENGTH OF LIFE OF THE ADULTS AT A CONSTANT TEMPERATURE AND DIFFERENT RELATIVE HUMIDITIES

No great variation in the length of life of the adult grasshoppers occurred in these investigations, in spite of the fact that the relative humidity was maintained at 40, 50, 60, 70, 80 and 100 per cent in the different experiments (Table III). However, the cause for the apparent lack of variation might be sought in the meagerness of the data that was available for our experiments. If we are justified in drawing conclusions from the small number of experiments conducted, then we must conclude that a relative humidity of 100 per cent shortens the life of the adult grasshoppers materially. On the other hand, the optimum relative humidity was not indicated through our experiments. Throughout our experiments the female grasshoppers outlived the males with which they were confined, but as soon as a male died, another male was added to the container in which the female was located. The following table shows the duration of life of the female grasshoppers used in our investigations.

TABLE III. - Length of life of female grasshoppers kept at a constant temperature of 27° C. and at relative humidities of 40, 50, 60, 70, 80 and 100 per cent.

Per cent relative humidity	Jar number	Length of life in days	Average length of life in days
40%	1	12	
	2	12	
	3	17	13 2/3
50%	1	12	
	2	9	
	3	12	11
60%	1	13	
	2	17	
	3	9	13
70%	1	17	
	2	9	
	3	9	11 2/3
80%	1	9	
	2	8	
	3	12	9 2/3
100%	1	10	
	2	8	
	3	7	8 1/3

OUTDOOR WORK

The purpose of conducting out-of-door work in this investigation was to secure data on the biotic potential of M. bivittatus maintained under these conditions. Such data could then be compared with data secured from cabinet work, and conclusions might be drawn relative to the efficiency of one or the other method. The out-of-door conditions to which our grasshoppers were exposed were as nearly identical to the natural environment of the grasshoppers as it was possible to make them. In spite of this, it must be remembered that it was necessary to confine the grasshoppers in cages, which is a factor that is not normal but which cannot be avoided.

The method of studying insects in the out-of-doors but confined in cages is much older than is the cabinet method. Of late, however, the cabinet method is being used more and more, largely because the physical environmental factors can be controlled, and also because this work can be conducted throughout the year.

It is the opinion of the writer that the ideal method of carrying on investigations such as are discussed in this thesis would be through cabinet experiments, through out-of-door cage experiments, and finally through actual field observations where conditions are not disturbed by the experimenter. In this way single factors could be studied separately or in combination, the factors could be controlled as desired, and the objection of studying the insects in confinement could be checked at least to some degree.

CAGES USED IN OUTDOOR WORK

The cages that were used in our out-of-door work were cylindrical in shape, and measured 8 inches in diameter and 14 inches in height. The entire cages were made of galvanized tin and wire screen of 12 mesh to the inch. The cages were without bottoms; the lower portions were made of galvanized tin, while the upper parts were constructed chiefly of wire screen. A removable lid fitted over the top of the cage, and this also consisted chiefly of wire screen (Plate IV).

These cages had many advantages, the most important being the following:

1. Air would readily pass through the cages.
2. The cages could be placed over a small alfalfa or sweet clover plant and the base of the cages could be pushed into the soil without harm to the cages.
3. The tops of the cages could be readily removed for feeding and observational purposes.

The most serious objection to the cages was their small size, but since only a single pair of grasshoppers was ever confined to a cage at one time, this objection was not so serious as it might have been. Further, the grasshoppers soon accustomed themselves to being confined in the cages, and unless frightened, did not hop about to any unusual degree.

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PLATE IV. - Photograph of cages used to confine grasshoppers in outdoor conditions.

SOURCE OF MATERIAL

Grasshoppers which were used in our outdoor work were secured at the same time as were those that were subjected to cabinet conditions. Care was taken to see that they had not begun egg laying previous to confinement, and only such specimens were used as appeared normal and healthy.

LOCATION OF CAGES

The thirty-five cages used in the outdoor work were located on the college campus to the north of the college grove, in between two rows of shrubs about eight feet in height.

The purpose of choosing this space in preference to others was because it was close to our source of supplies, because some shade would be provided the cages, and because piped city water was available close by.

Before the cages were permanently stationed, the soil was dug up and the roots of grass and weeds were removed. Loose dirt was then packed in place of the sod removed, thus making this area somewhat higher than the surrounding soil. This eliminated the possibility of water collecting and standing in the cages and also made possible the collecting of egg pods without danger of breaking them.

Grasshoppers were placed in these cages on July 26 and here they remained until they died.

KIND OF WEATHER EXPERIENCED DURING THE PERIOD OF INVESTIGATION.

By weather, as used in this paper, is meant the natural meterological phenomena occurring in nature. These meterological factors unlike conditions found in cabinets are never constant for a day or even during many hours of a day.

In recording data on these studies, no attempt was made to keep a record of the weather conditions other than what would be noticed over a period by any casual observer.

The period of the year in which these grasshoppers were confined was very similar to that of the previous year. Both years were exceedingly dry when compared with the weather usually experienced at this time. Very little rainfall occurred while these studies were being made, and the temperature, though variable, was unusually high. A period of this kind is considered by most ecologists to be the optimum conditions for the development of grasshoppers. We must then, when studying the influence of these conditions, realize that a similar experiment in a different year might prove to be altogether different. Experiments conducted over a series of ten consecutive years, when averaged, should give a fair indication of the actual biotic potential of this species of grasshopper in an average year.

NUMBER OF EGGS LAID BY EACH FEMALE

No data was found in the literature read by the writer that gave information as to the number of eggs that may be laid by M. bivittatus. Riley (10) and Parker (1) both conducted experimental work to determine the number of egg pods that may be laid by M. mexicanus, and the results obtained by each worker varied greatly. Riley found that only in a few cases did the number of pods reach as many as four and more commonly two were as many as would be obtained. Parker, on the other hand, found that a single female kept under favorable conditions in a cabinet might lay as many as 15 pods, the average number of pods laid by thirty caged females being 8.8 pods per female.

The total number of eggs per pod was not recorded in either the work done by Riley or Parker. However, Parker stated that in most of his work the number of eggs per pod ranged from 12 to 36 eggs, but that the average was no higher for females laying a few pods than for those laying many.

Experiments to determine the number of eggs which would be laid by thirty-five female M. bivittatus grasshoppers were conducted by the writer in the summer of 1931. Pairs of this species were collected in the field in July and caged as already described. Upon the death of these individuals, a count was made of the number of egg pods laid by each female, and following this the egg pods were carefully broken open and the number of eggs in each egg pod was counted.

The egg pods and eggs laid in 27 of our experimental cages were counted, but the pods from the remaining eight cages were left in a dry environment, with the consequence that the eggs dried so that it was impossible for a count to be made of the eggs.

It must be remembered that the grasshoppers used in this experiment were collected in the field before they had begun egg laying, that the grasshoppers were fed and watered daily, and that they were given conditions as favorable to them as it was possible to make them.

The number of egg pods laid by each female grasshopper and the number of eggs laid by each individual is recorded in Table IV. It will be noted that one grasshopper laid as many as 441 eggs, that the smallest number of eggs laid by any one grasshopper was 97 and that the average number deposited was 232.2 eggs per female.

NUMBER OF EGG PODS LAID BY EACH FEMALE

The number of egg pods laid by each female grasshopper under the caged outdoor conditions to which the insects were subjected varied from two to six, with an average of 3.6 pods per female (Table IV). It should be remembered, however, that the season during which these experiments were conducted was hot and dry and that the grasshoppers were surrounded with an environment that was highly favorable to them. Consequently, there might have been a difference in the number of pods and eggs laid by caged grasshoppers and those leading a free life in 1931. Further, conditions were made very favorable to the caged grasshoppers and this circumstance undoubtedly increased the normal span of life of the grasshopper and this, in turn, might have increased the number of egg pods laid as well as the total number of eggs.

TABLE IV. - Number of days lived by each adult female and the number of egg pods and eggs laid by each in outdoor experiments.

Cage number	Number of days of adult life	Number of egg pods laid by each female	Number of eggs laid by each female
1	56	2	123
2	56	6	441
3	34	4	220
4	50	4	217
5	63	6	373
6	48	4	263
7	47	3	183
8	60	4	358
9	52	3	248
10	57	4	295
11	49	3	189
12	35	3	108
13	51	4	365
14	54	4	178
15	41	2	133
16	31	3	147
17	55	4	207
18	49	4	321
19	53	5	343
20	53	2	97
21	48	3	117
22	57	4	210
23	55	3	187
24	50	3	211
25	58	2	115
26	48	4	302
27	31	5	323
Average	49.2	3.6	232.3

THE LENGTH OF LIFE OF ADULT GRASSHOPPERS UNDER OUTDOOR CAGED CONDITIONS.

It was thought possible that there might be a direct correlation between the length of life of adult female grasshoppers and the number of egg pods and total number of eggs which such females might lay. Accordingly, it was decided to keep an accurate record of the length of life of each female in our experiments and the number of eggs and egg pods laid by her.

In the summer of 1931, 35 pairs of M. bivittatus were collected in the field on July 14 and caged out-of-doors. Here they were kept until they died. Records were obtained from only 27 of the 35 pairs, however. The average number of egg pods per female for these 27 pairs of grasshoppers was 3.6 pods (Table IV). The average length of life of these female grasshoppers was 49.2 days. The shortest period lived by any female was 31 days, while the longest period was 63 days.

It was decided to divide the 27 female grasshoppers into four groups, basing the grouping upon whether the grasshoppers lived 30 to 39 days, 40 to 49 days, 50 to 59 days or 60 to 70 days (Table V). It will be noted that the first three groups did not differ materially in the average number of egg pods laid, but that the total number of eggs laid per individual increased the longer the group survived. However, because of the small number of individuals worked with, it would be advisable to repeat this work before definite conclusions are to be drawn.

TABLE V. - Female grasshoppers caged under outdoor conditions, and grouped into four classes, depending upon longevity, the average number of egg pods laid per female for each group and the average number of eggs laid per female for each group.

	Average number of egg pods laid by each female	Total number of eggs laid on an average by each female
Group living 30 to 39 days	3 3/4	199.5
Group living 40 to 49 days	3 3/8	210.7
Group living 50 to 59 days	3 3/10	235.3
Group living 60 to 70 days	5	365.5

SUMMARY

(1) M. bivittatus (S.) or the two-striped grasshopper is one of the most prevalent and destructive species occurring in the northwestern plains states.

(2) A study of the influences of temperature and humidity upon the biotic potential of this species was instituted as a means of securing data, whereby a prediction could be made as to their expected abundance following years of known conditions of weather.

(3) Cabinet methods were used to make possible longer continual studies of environmental factors upon this insect.

(4) To secure and maintain constant environmental conditions in the cabinet at all times proved difficult.

(5) Of the factors experimented with, controlled humidity offered the most difficult solution.

(6) A means of rearing young grasshoppers to maturity was never satisfactorily solved.

(7) Specimens for study were collected before egg-laying began.

(8) Since a constant temperature of 27° C. was the only temperature worked with in our cabinet experiments, it is impossible to state that different constant temperatures will effect the reproductive potential of this grasshopper differently.

(9) An attempt was made to determine what effect relative humidities of 40, 50, 60, 80 and 100 per cent would have upon

the reproductive potential of M. bivittatus. The data obtained was not sufficient to warrant us drawing any definite conclusions concerning the influence of these humidities.

(10) The length of life of the adult female grasshoppers kept in a cabinet maintained at 27° C. and at 40, 50, 60, 70, 80, and 100 per cent relative humidities, did not vary materially. In general, the female grasshoppers lived somewhat longer than the males.

(11) Outdoor experiments were run to determine the reproductive potential of M. bivittatus under conditions as nearly normal as it was possible to make them.

(12) The cages used in outdoor work were more satisfactory than were the containers used in the cabinet experiments.

(13) Specimens of grasshoppers for our outdoor work were obtained before egg-laying began.

(14) The cages were so located that they were partly shaded during some periods of the day.

(15) Weather conditions at the time the experiments were being studied were unusually warm and dry.

(16) The number of eggs laid per female varied from 97 to 444.

(17) The number of egg pods secured from each cage varied from 2 to 6.

(18) The length of life of grasshoppers in outdoor conditions was much longer than under cabinet conditions.

(19) The total number of eggs laid by grasshoppers kept under outdoor conditions apparently varies with the length

of time the female grasshoppers survive, the shorter the life period, the fewer the number of eggs that are laid, and vice versa.

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