A STUDY OF DEVELOPMENT OF THE ANGOUMOIS GRAIN MOTH ON VARIOUS FOODS

THESIS FOR THE DEGREE OF M. S.

James M. Merritt

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CONTENTS

INTRODUCTION

The Angoumois Grain Moth (*Sitotroga cereallela*, Olivier) has been known for many years as a pest of grains in the field and in storage, (29), (13), and because of its economic importance the field biology has been worked out from time to time by various authors, (1), (3), (5), (6), (7), (8), (17), (19), (20), (21), (22), (25), (28), (31), (30).

Back, (2), in discussing the grain moth as an economic pest, says that, "Under ordinary climatic conditions ......, temperature seems to be the most important controlling factor in development."

Back (2) also reports that, "The Angoumois grain moth has been bred from wheat, barley, oats, buckwheat, corn, sorghum, milo, rice, beans, chickpeas, and cowpeas. It is a general feeder upon all seeds of the cereal type. It causes greatest loss to the wheat and corn in this country, though instances of serious attack are recorded frequently upon other grains." This is substantially in accord with the statement of

Girault (17) that, "The grains attacked are wheat, corn, and other cereals less seriously." Other authors report that rye, popcorn, Sudan grass, and possibly grass seed are attacked, (6), (23), (10), (28).

According to Back (2), the moths may lay as many as three hundred eggs apiece, although one hundred-fifty is probably a fair average. King (25), also working on the field biology of the grain moth in Pennsylvania, arrived at approximately the same conclusions.

Recently, (11), (9), (24), this insect has been selected as the host for the large scale production of the tiny Hymenopterous parasite, _Trichogramma minutum_ Riley, for use in biological control of various insect pests. The use of the Angoumois grain moth was recommended by S. E. Flanders (11). Among the advantages evidenced by the research of Flanders and others, (9), (24), were the multivoltine type of life history, (4), high biotic potential, (4), and an environmental resistance made up of factors that could be controlled effectively by laboratory methods.

OBJECT OF EXPERIMENTS

The following experiments were carried out in the study of the development of the Angoumois grain moth on various foods, to determine the effect of this factor on the partial potential*, and were designed to enhance, without distortion, variations due to food to a point where they could be measured and compared.

STUDY OF LITERATURE ON PARASITE PRODUCTION

Summary of Methods

The procedure in the utilization of the Angoumois grain moth as a host for the production of *Trichogramma minutum* is to build up a large colony of the host, from which eggs are obtained. These are made available for the oviposition of the parasites, and in this way a large colony of

*Chapman, (Chapman, Royal N., Animal Ecology P. 186), defines the "general term 'biotic potential'" as "a quantitative expression of the dynamic power of the species which is pitted against the resistance of the environment in which it lives in its struggle for existence". He further says that because of the "serious difficulty ...... in determining ..... this absolute biotic potential ...... There is a practical advantage in introducing the term 'partial potential' to represent the biotic potential of a species under a given set of conditions".

parasites is obtained, from which liberations are made for the control of other insect pests, (9), (11), (12), (13), (14), (15), (24), (26), (27), (34), (35). This procedure has been highly developed, the optimum temperature and humidity for the development of the moths has been computed, (11), (12), (19), (20), (21), (22), and very large colonies have been produced, (14), (15), (27), (24), (34).

Factors Affecting Efficiency

However, Flanders (15) in a climate approaching the optimum for the development of the grain moth, reports that an infestation of *Plodia interpunctella* increased to become a very serious competitor and very materially reduced the efficiency of the moth colony in the production of eggs.

List, Daniels, and Bjurman (26) reported a like reduction in the efficiency of a colony upon the use of grain contaminated with the harvest mite (*Pediculoides ventricosus*).

Wishart (34) developed elaborate breeding methods patterned after Flanders' (14), but modified to suit a more northern climate. His

results (34) show that the production of moths by these methods was limited by the difficulty encountered in providing, in a manner optimum for the development of the moths, a food supply uncontaminated by competing colonies of insects or mites.

The results of the experiments previously reported,(15), (26), (34), show that the greatest difficulty in the production of an unlimited number of Angoumois grain moths under controlled conditions of temperature and humidity is the maintenance of an optimum food supply. In other words, when reared under laboratory conditions, that part of the environmental resistance caused by temperature and humidity can be governed, being only a matter of technique. The factors controlling the efficiency of the colony are those concerned with the proper food or media furnished the moths.

The host list for the grain moth, as reported by Back (8), and others, indicates a possibility that under uniform conditions except as regards food, some preference among the different grains might be exhibited. Wheat and corn, for this reason, may be classed as preferred hosts, from an economic status. The data on hosts preferred by the moths when reared in the laboratory is limited. Wishart (34) reports that he abandoned the use of wheat in the production of moths and substituted corn, because of the resulting increase in size of moths. List (27) tried some ten different grains and decided that corn and wheat were the most practical. He says that the larvae seem to prefer the wheat, or they are able to make entry into this grain easier, but records that the moths produced are smaller.

Therefore, if there are inherent factors in the grain exposed for infestation by the grain moth which affect the partial potential, explanation could thus be made of variations in infestation of different grains which occur in the field. Also, as it has been shown that the efficiency of a colony of moths reared in the laboratory is governed by the partial potential which is attained, it is obvious that any factor affecting this would be very important, multiplied as it is by the multivoltine type of life history.

PROCEDURE

Testing of Possible Methods

The first problem was that of maintaining colonies of Angoumois grain moths, under laboratory conditions, of sufficient size to conduct the desired experiments, but not yet so large as to require elaborate equipment. A flexibility of the population maintained was necessary far in excess of that required for commercial laboratory production.

As has been stated, the methods of maintaining large colonies have been developed in detail by various experimenters, (9), (11), (12), (13), (14), (15), (24), (26), (27), (34). A study of these methods seemed to show that by reducing the scale of operations it would be possible to develop a method of handling a small colony by the same technique, even under experimental conditions. Therefore, a detailed study of the various methods was made, and such of these as seemed adaptable were tested on a small scale. These are reported, with a summary of the original method, and the results obtained in testing their adaptability to these experiments.

The method used by Wishart (34) was to rear the moths in a cabinet having a tier of trays on one side sloping at a slight angle toward the side, so that the front of the trays were highest and opened into the cabinet. The trays were separated by strips of wood which left one-

eighth of an inch between them for the escape of the moths. From the open space in front of the trays, moths were collected daily by means of a vacuum hose and were transferred to oviposition cages.

The oviposition cage used was cylindrical and closed on the ends by 20-mesh screen, through which the moths laid their eggs when the cage was placed on end in a dish of cornstarch. The eggs were separated from the starch by sifting through a 60-mesh bolting cloth.

These rearing cages were obviously not adapted to rearing small colonies of moths, but the apparatus used in oviposition was tested. A few moths were confined in a small cage constructed in the same way (plate 1). Eggs were obtained in this manner, but it was found that the 60-mesh bolting cloth was too coarse to separate the eggs from the starch. Single eggs readily passed through while such difficulty was experienced in sifting the starch through, because of the tendency to form lumps, that it was evidently impossible to use a finer grade of cloth. This was probably due to the high humidity in the breeding chamber, but it was decided that any humidity low enough to keep the starch from becoming lumpy would be undesirable in the production of moths. The method was discarded

as being too inaccurate on the scale desired.

Another method that evidenced a possibility was that recommended by Flanders (15), who used a cone of metal, eight feet in diameter, with the sides sloping at an angle of 22. 5 degrees for a rearing chamber. The moths left this cage through an opening in the top six inches across, and were collected in a cage. The cone was filled with corn, then raised one-eighth inch to allow the moths room to crawl out over the corn. Moths obtained in this way were induced to lay eggs in the customary way.

This type of breeding cage was duplicated on a very small scale (Plate 2), using a cone of the same angle about ten inches across, but it was found to be very unsatisfactory. The moths did not emerge freely from the cone, and upon examination it was found that the moths were unable to use more than the surface of the grain to any advantage. Thos emerging in the deeper parts of the cone were unable to crawl to the surface. An added disadvantage of this method was that observation of the moths was impossible.

Flanders (12), also developed an oviposition cage, consisting of a smooth cylinder capped on each end with a 20-mesh wire screen.

Moths were confined in this and stimulated to oviposition by the crevices formed by their bodies and by the passage of a current of air up through the cage.

This was combined with the method just described (Plate I), not so much to test its use as a device for measuring the oviposition rate as to see if the moths would oviposit readily this way. A cylindrical cage, covered with an inverted cone, was filled with infested grain. The cone was smaller than the diameter of the cylinder, so the moths could escape around the cone through the aperture at the top. The moths were forced to descend through the cone to another cylinder, capped at the end with 20-mesh screen, by the action of a current of air passing down through the cone, and the eggs were laid through the screen.

Oviposition was readily secured in this manner, but considerable difficulty was encountered in separating the eggs from the dead bodies of the moths. The dead moths could not be separated from the live ones continually emerging, and accumulated in the end of the cage. The apparent possibility of premature death of the moths under these conditions, as well as the inaccuracy of data relative to the number of eggs laid by each

moth, prevented the use of this method in these experiments.

Another important part of the methods cited, (24), (15), (34), (27), is the use of an adaptation of a vacuum cleaner to move the moths from cage to cage. This was done by using the current of air to pick up the moths and transport them to the next cage, where they were removed from the air stream by baffles. The use of this system has been necessary in the commercial work, but the smallest type of machine which could do the work would be needlessly costly and elaborate in these experiments. In the selection of a breeding cage this had to be considered, and a substitute devised.

These experiments were carried out in a cabinet designed to facilitate the maintenance of constant temperature and humidity. This was constructed in the College carpenter shop.

The cabinet was built two feet deep, four feet high, and eight feet long. The frame is pine, two inches square, and is covered with Nu-Wood, a material of considerable insulating value. The box rests on one of the long narrow panels.

Access to the interior is by means of three large doors, each two feet, eight inches wide and four feet high;and three small doors, each set

in one of the large ones. The small doors are fitted with glass windows to allow observation.

The interior is divided into two parts by a horizontal shelf extending almost the full length of the box. Heat is supplied by two electric elements in one end, controlled by a centrally located thermostat. The humidity is governed by the installation of a nitrogen-filled bulb of the proper size, partially submerged in a pan of water located under the heating elements. Circulation of air is afforded by spaces left at either end of the shelf, and insured by the installation of a fan. This was necessary because of the length of the cabinet, which is so great that it precludes efficient circulation by convection currents alone, allowing condensation if the temperature falls to the dewpoint in the cooler portions. A temperature of 80 degrees F. and a relative humidity of about 70 per cent was maintained.

The requirements for a rearing cage for this work were that it should accomodate an amount of grain sufficient for the development of the colony without the possibility of lack of food; that the grain should be readily available for entrance by the young larvae; that it allow the ready emergence of the adult moths; and that observation of

the colony be easy. It was also necessary that the temperature and humidity maintained in the cabinet could be readily and equally maintained in the cages.

The availibility of a supply of glass plates, five by seven inches, of uniform thickness prompted the construction of the rearing cage (Plate 4), which was used in this work. The cage was built with these glass plates for the top, bottom, front and back. They were held in place by the ends, which were of "Prestwood", grooved near the edges to accomodate the ends of the glass plates. The top and bottom plates were set in to allow the fron t to slide up in the grooves giving access to the interior. The glass plates were fastened in place with linoleum cement, which was allowed to thicken and then applied like putty in the corners.

Air circulation, necessary to keep the cage conditions equal to those in the cabinet, was afforded through circular openings in each end, three and one-half inches across, which were covered with 40-mesh screen. When these cages were set one above the other across the cabinet and the air circulation controlled by a fan, the velocity of the circulating air varied little if any between the cages, thus insuring equal control of temperature and humidity.

While the larvae of the Angoumois grain moth are reported to develop normally if there are as many as four in a single grain of corn (16), only one or two are produced from a single grain of wheat. Therefore, there being about four hundred kernels of wheat in one cubic inch, ten cubic inches should be ample for the development of the eggs of twenty females, or three thousand larvae (2).

In the cages just described, ten cubic inches of grain covered the bottom of the cage to a depth of about one-third inch, making all the grain available to the larvae, and facilitating the emergence of the adults, since they were not hindered by having to crawl through any amount of grain to reach the surface.

The use of a cage of this type necessitated a system that did not require that the live adults be handled, as there was no provision made for the use of a vacuum apparatus. This was accomplished by handling the individuals in the pupal stage. This prevented damage to the moths in handling, insuring their uninterrupted natural development. The pupal stage was readily separated from other stages by the presence of the emergence disc on the kernel, which is formed just before the larvae pupate. Isolations to obtain virgin females were also possible by this method.

Eggs of the Angoumois grain moth were secured from the New York Agricultural Experiment Station through the courtesy of Derrill M. Daniel. These were reared for several generations on corn, and a fairly large colony developed. One cage, however, was crowded out by an infestation of the lesser grain borer, Rhizopertha dominica, which had been introduced in the grain. The colony of moths on corn was continued throughout the experiments as a stock supply.

The following experiments were set up to determine the effect of various foods on the development of the moths. The points selected for investigation were effect on the sex ratio; effect on size of moths produced; and resultant effect on the partial potential of the various colonies.

Colonies were started on corn, wheat, oats, buckwheat, and beans. Later colonies were started on sorghum and Sudan grass. These grains were selected because they represented the variations in size of the grains that are recorded as being attacked by the grain moth.

The grains were sterilized in an autoclave at fourteen pounds pressure for twenty minutes to eliminate any insect or mite infestation that might be present.

A measured amount of each grain was put into each cage and a known number of grains of corn infested with the Angoumois grain moth was introduced into each cage to act as an inoculation.

When these moths died, they were removed and the number of each sex recorded.

The breeding cages were kept under close observation, and when the female moths began to emerge, sufficient pupae were removed from the cage to inoculate another cage in the same manner as the first. The rest of the female moths were allowed to emerge at will. At intervals they were anaesthetized and removed from the cage, those from each cage being isolated for study in a separate vial in picklene.

When all the moths had emerged, the grain was discarded. This helped to control the infestation of mites, which becomes a serious difficulty if the grain is used for more than one generation. The possibility of this method for control of mites has also been suggested by List (27).

This procedure allowed the continuance of the experiments for as many generations as desired without varying the cage conditions, and the quantity and quality of grain could be maintained uniformly.

DATA

Method of Collection

After the moths from each cage were isolated, detailed observations were made as follows: Three hundred moths from each cage were measured and the sex ratio determined. This was taken as an average for the test. Measurement of the length of the moth was made with the wings folded, from the front of the head to the end of the wings. This permitted a more accurate comparison than weight, as the difference in size of the bodies of the males and females would affect the weight, as would variations of moisture content of the dead moths. Counts were also made of the total number of moths emerging from each cage to determine the partial potential of the colony.

Results

The results of the observations of the colonies to determine the variation in size, sex ratio, and partial potential are recorded in Tables I, II, and III:

Table I - Length of Moths, (mm.)

	1st Generation			3rd Generation		
	Female	Male	Average	Female	Male	Average
Corn	6.455	5.775	6.115	7.044	6.403	6.724
Wheat	5.111	4.719	4.915	5.880	5.682	5.780
Oats	4.960	4.707	4.834	5.509	4.971	5.213
Buckwheat	4.925	4.672	4.799	5.173	4.765	4.969
Sorghum	4.864	4.450	4.657	--	--	--
Sudan Grass	4.240	4.226	4.233	--	--	--

Table II - Sex Ratio

	1st Generation	3rd Generation
Corn	145:155	177:141
Wheat	149:151	173:163
Oats	147:153	59 : 91
Buckwheat	138:162	92 :108
Sorghum	140:160	-----
Sudan Grass	156:144	-----

Sex ratio of 2804 moths, 1:1.038

Table III - Number of Adults
Produced Per Generation.

	1st Generation	3rd Generation
Corn	40.4	47.0
Wheat	134.9	129.5
Oats	65.7	*25.46
Buckwheat	93.9	36.66
Sorghum	85.94	---
Sudan Grass	100.63	---

DISCUSSION OF RESULTS

The results in Table III were subject to considerable experimental error, because any mortality of the larvae during the period of development would affect it, as would also the production of any sterile eggs. However, it seemed that no more quantitative method was compatible with the conditions of the experiment, and the results would be indicative of what might be expected if the work was repeated on a larger scale.

During the course of the experiments, difficulty was encountered in controlling factors which affected the experimental error. It was found that over-sterilization of the grain caused a high mortality of the larvae, and in the case of beans inhibited all development. This was also true of oats when they were twice sterilized. All grains were affected to some extent, so steam sterilization had to be abandoned in the control of other insects and mites. The test on beans was abandoned, while the test on oats was continued by the inoculation of a new supply of grain with moths from the stock cage. In all other cases there was sufficient emergence from the over-sterilized grain to allow the experiment to continue.

The difficulty encountered with sterilized grain detracts from the value of the results, but the observations on the size and sex ratio of moths produced were made on a number of individuals sufficient to give veracity to the results. That is, observations of size and sex ratio of moths from one kind of grain, made on the first generation, then again on the third generation, if all had been subject to the same environmental conditions, would have a value equal to that obtained by making observations on all three generations if enough individuals were examined.

The data in Table I shows that the females produced during the course of the experiment averaged .379 mm. longer than the males, as might be anticipated. The data, as recorded in Table I, also shows that these results were uniform, in that in all cases the females were slightly larger than the males.

The largest moths were produced on corn, and the difference in size of these and any moths produced on the other grains was striking. The size of the moths reduced quite regularly on the different grains, in the following order: Corn, wheat, oats, buckwheat, sorghum, Sudan grass. The variations in size, except for the moths produced on corn, was not particularly large, but the results obtained from the third generation are in accordance with those of the first generation. The moths produced in the third generation were slightly larger than those of the first. This difference is so uniform as to be significant, but could only indicate that the effect on size as reported is temporary, and further research would be required to settle this question.

Sufficient variation in size is caused by different grains to justify the observation of Wishart (34), and List (27), that judging from the size of moths produced, corn was a better host than wheat for the commercial production of Angoumois grain moth.

The determination of the sex of the moths (Table II) from each colony was made when they were measured, and in this way the sex ratio was determined for 2304 moths. The ratio computed was 1:1.038, and while the males slightly outnumber the females, this figure is in accordance with the sex ratio as reported by Back (2). The sex ratio as determined for the different colonies is subject to considerable variation.

The variation from 1:.919 in the case of the colony reared on corn to 1:1.185 in the case of the colony reared on oats, is not sufficient to effect the efficiency of a colony reared on any of the grains tested. The variation is not correlated with the size of the grain, and it is possible that by using larger samples it would be eliminated, as it was when the results were averaged to obtain the ratio given above.

The results in Table III are, as has been pointed out, subject to a larger experimental error than those in the previous tables. Certain deductions are justified by the results tabulated, however. In the case of the figure given as the third generation result of the colony on oats, as marked by an asterisk, moths from the stock cage were used as inoculum, actually giving the figure a value comparable to that of the first gneration. It is listed under the third generation because the cage conditions for the figures in that column were alike. The other figures represent results of observations made on the first and third generations of a colony bred for three consecutive generations on the same grain, each cage inoculated from the one previous, as has been described.

The results show that certain of the grains attacked by the Angoumois grain moth are more suitable for the development of the moth than others. However, Table III shows that there is no reason to believe that any correlation exists between the size of the grain and the increase in population of a colony of moths reared upon it. The results obtained from colonies reared on corn and wheat are respectively very comparable. However, three times as many moths were produced in the wheat colony. The reason for this is shown by a consideration of the data of Tables I and III taken together.

Table I shows a definite correlation between the size of the moths and the size of the grain on which they were reared, and this might be taken to indicate that there would be a correlation between the size of the grain, the size of the moths and the number of eggs laid, particularly as the moths are reported to lay a varying number of eggs, (2). Table III contradicts this by showing no correlation between the size of moths and the number of adults produced per moth.

CONCLUSIONS

The obvious conclusion is that some inherent factor, structural or physiological, irrespective of size, governs the desirability of the grain for the development of the moths. The first generation results in Table III are all produced by inoculating the various cages with moths from the same cage of corn. Approximately the same number of eggs should have been laid in each case, yet there was a variation of nearly one hundred in the number of adults produced. List (27) says that "The larvae seem to prefer wheat to the corn, or else they are able to make entrance into the grains more readily". These experiments show that the larvae were able to gain entrance into the grains with varying ease. No decrease in size was found in the moths produced in the third generation on corn as compared with the first, yet the number of moths produced was the same. Moths from the same colony produced three times as many when reared on wheat, and continued to do so. Even when reared on Sudan grass, where the inhibition of growth as evidenced by the smaller size of adults was greater, two and one-half times as many were produced. Therefore, the assumption is reasonable that: regardless of the size of moths or the number of eggs laid, the number of moths reaching maturity is dependent on the ability of the young larvae to enter the kernel, in which the development is governed by the size of the grain, at least insofar as the grains tested in these experiments are concerned.

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PLATE 1 -

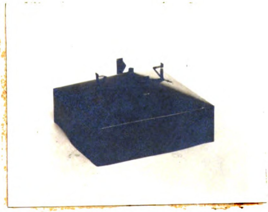

PLATE 2 -

PLATE 3-

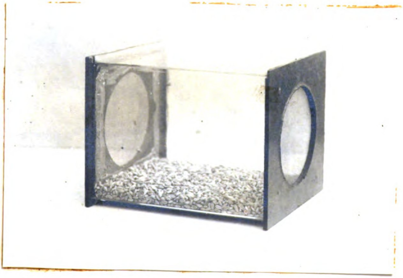

PLATE 4-