THE BIOLOGY OF *PTINUS VILLIGER* (REIT.)

A Thesis

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by

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ABSTRACT

Laboratory experiments were carried out to study the development of the hairy spider beetle, Ptinus villiger (Reit.) on four foods at three temperatures and three relative humidities. Eggs were reared individually on whole wheat, bran, ground wheat, and vitamin enriched flour since these materials are typical of stored commodities infested by the hairy spider beetle.

Insects were reared at 48°, 68°, and 80°F., and at 40, 60, and 80 per cent relative humidities. In most cases 36 replicates were used for each food, temperature and relative humidity. Insects were examined as often as possible to record significant observations and life cycle changes. In this way an accurate life history was kept of each individual insect as it progressed through the larval and pupal stage and emerged as an adult. Newly emerged adults were weighed and sexed; some were replaced for further observation. The majority were paired and exposed to low temperatures in an attempt to induce egg laying.

Generally, temperature had the greatest effect on development. Relative humidities of 60 and 80 per cent had no significant effect on development. Ground wheat and bran were significantly better for development than whole wheat kernels and vitamin enriched flour. Some larvae went into diapause at each temperature and humidity. More larvae went into diapause on flour at 60 and 80 per cent relative humidity when the

temperature was 68°F. Adults subjected to 35°F. showed no ill effect. They laid no eggs at 35°F., 48°F., 68°F., and 80°F.

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INTRODUCTION

The present work comprises an attempt to evaluate the effect of food, temperature, and relative humidity on the life cycle of the hairy spider beetle, Ptinus villiger (Reit.). This information can be used to predict the areas where the species could exist and to compare them with the existing world distribution. Also, and of economic importance, the results of this study may be used to decide whether P. villiger is likely to become a major pest in any particular place. During the past twenty years serious damage has been reported by flour dealers, storekeepers, and milling companies, particularly in the Prairie Provinces. One of the main objects of investigating the biology of insect pests of economic importance is to determine how rapidly the insect population will grow. The figures obtained normally include the duration of development of the species reared on particular foods at several temperatures, and relative humidities, at each stage of life. In addition, the effect of these conditions on egg-hatching, mortality, and incidence of diapause will be evaluated. Lastly, adults obtained will be subjected to various temperatures in an attempt to find out preferred temperatures for ovipositing.

The principal form of spider beetle damage is the lowering of quality due to the presence of live or dead insects, excreta, and cocoons. The nutritional value of food may readily be affected by the presence of large numbers of the insect. Besides the actual damage caused by the insects in the infestation of stored food products there is the psychological effect of the customer's aversion to contamination of any kind in such products. The layman has been educated to regard all foreign matter in food as dirty and unwholesome Financial losses may result from loss of patronage and from the necessity for salvaging infested cereals or selling them at reduced prices.

Zacher, in 1939 claimed Ptinus villiger (Reit.) to be an eastern Siberian species. It spread to Canada possibly via the United States in 1915 and to Silesia and Germany in 1931. Earlier, Hellēn in 1925 had recorded it from Finland. In recent years it has been found occasionally at British ports on Canadian produce, especially flour, but also many other foodstuffs.

Gray (1933) pointed out that the hairy spider beetle occurs in practically the entire list of foodstuffs handled by milling companies. This includes flour (patent, whole-wheat, graham and rye), cornmeal, rolled oats, oatmeal, farina, as well as feeds such as bran, shorts, and various meal preparations. It is also able to feed in wheat.

In 1932 an elevator company encountered these insects in its sample boxes at Morden, Manitoba. The same condition was found in ten other elevators, two of which showed some signs of infestation in the bins. The use of country elevators for storage purposes over long periods of time, coupled with the ability of this insect to live in grain, constitutes a potential threat to grain in country storage.

Smallman and Gray (1948) have pointed out that in Manitoba the adult beetle commences egg-laying in mid-April. It continues for three months, reaching its peak of oviposition in mid-July. Many larvae complete their feeding during the summer. Some form a pupal cocoon, pupating and becoming adults before the winter. Others pass the winter as free larvae. Generally those adults emerging in the autumn do not lay eggs until the following spring. Thus it appears that either the adult beetles require an exposure to low temperatures before they can lay eggs or the pre-oviposition period of sexual maturation is comparatively long.

Observations in the laboratory indicate that the pre-oviposition period of sexual maturation is relatively long. Gray (1933) considers P. villiger to have only one generation per year in Canada. Indeed some individuals may require two years for development before ovipositing, spending one winter as a larva and the next one as an immature adult.

Gray and Watters (1954) stated that spider beetles are the most important insect pests of flour storage warehouses in Canada. Of these, several species occur in cereal warehouses, the hairy spider beetle, P. villiger (Reit.), being the most predominant. It was found that products that sell slowly and tend to remain in warehouses from one year to the next could become heavily infested and serve as a source of infestation.

MATERIALS AND METHODS

Eggs were obtained from two sources:

1. Laboratory cultures set up with adults captured from warehouses in May, 1956.
2. Test sacklets containing flour set up as oviposition sites in infested warehouses. These two-pound sacklets were collected after an exposure of approximately three weeks and their contents sifted for eggs.

Generally South-Western Manitoba was the main site of these warehouses.

Eggs were incubated in cubicles $\frac{1}{2}$ inch in diameter and $\frac{1}{4}$ inch deep. These cubicles were made by pressing six circular depressions in 1/64 inch acetate sheeting cut in specially designated sizes of three inches by one and five-eighths inches. In this way they could be stored upright in microscopic slide boxes. These boxes were cut in half, each half able to hold six acetate strips, or the sum total of thirty-six cubicles. Each cubicle was used as an individual rearing cell, one egg being placed on food in each cubicle.

Two slide boxes, each containing thirty-six cubicles, were stored in a small desiccator. Four slide boxes were stored in a large one. The reason for such a set up was both practical and time-saving. In this manner six replicates could be quickly and accurately examined under a binocular microscope. Figure 1 shows a diagrammatic plan of a group of six cubicles on acetate sheeting. Figure 2 shows how six of these groups fit into a slide box referred to as a lot. Figure 3 illustrates four lots in a large desiccator. Figure 4 shows two slide boxes in a small desiccator.

Eggs obtained for cultures were put into 48°F. incubation rooms if they could not be used the same day. This slowed down their hatching process. When ready they were placed on the food in the cubicles with a soft camel's hair brush. Only eggs that looked viable were used. Generally viable eggs showed a characteristic pearly opalescence. Non-viable eggs usually were of a dull brown color or wrinkled. Newly hatched larvae were able to feed on the food immediately.

Insects were reared at 80°F. in a constant temperature cabinet. This cabinet was fitted with an electric fan to maintain air circulation. Two germination rooms kept at 68°F. and 48°F. were used to rear insects at those temperatures.

The humidity was controlled at 40, 60, and 80 per cent. This was accomplished with desiccators containing sulphuric acid and water solutions mixed in appropriate proportions as outlined by Solomon (1951). For a relative humidity of 40 per cent, 30.1 ml. of sulphuric acid were added slowly to 50 ml. of water. Nineteen ml. of sulphuric acid slowly added to 50 ml. of water gave a relative humidity of 60 per cent. Eleven and one-third ml. of sulphuric acid slowly added to 50 ml. of water gave a relative humidity of 80 per cent. The amount of solution required for small desiccators was 200 ml. The amount of solu-

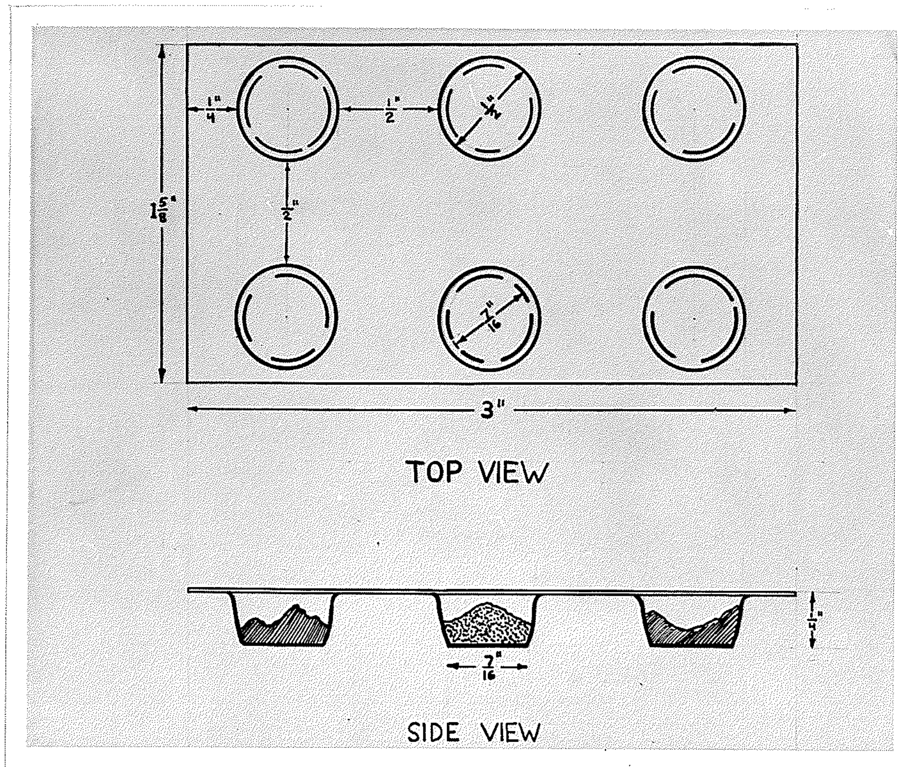

Figure 1. Diagrammatic plan of a group of six cubicles on acetate sheeting.

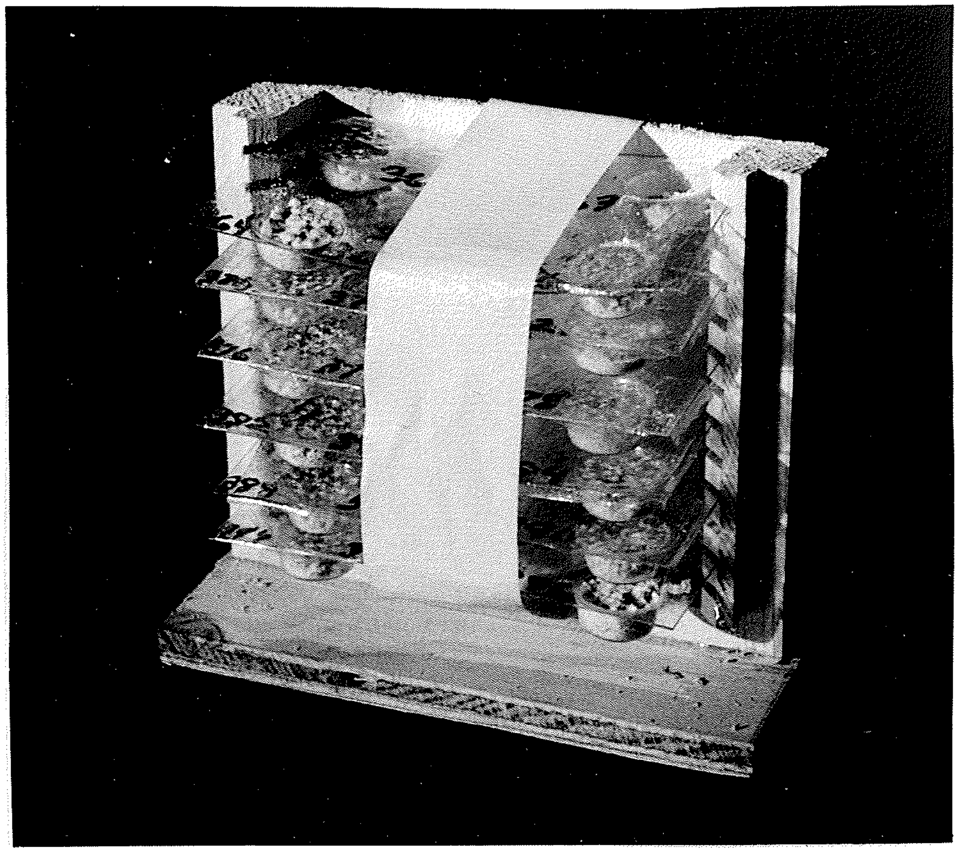

Figure 2. A lot of 36 replicates.

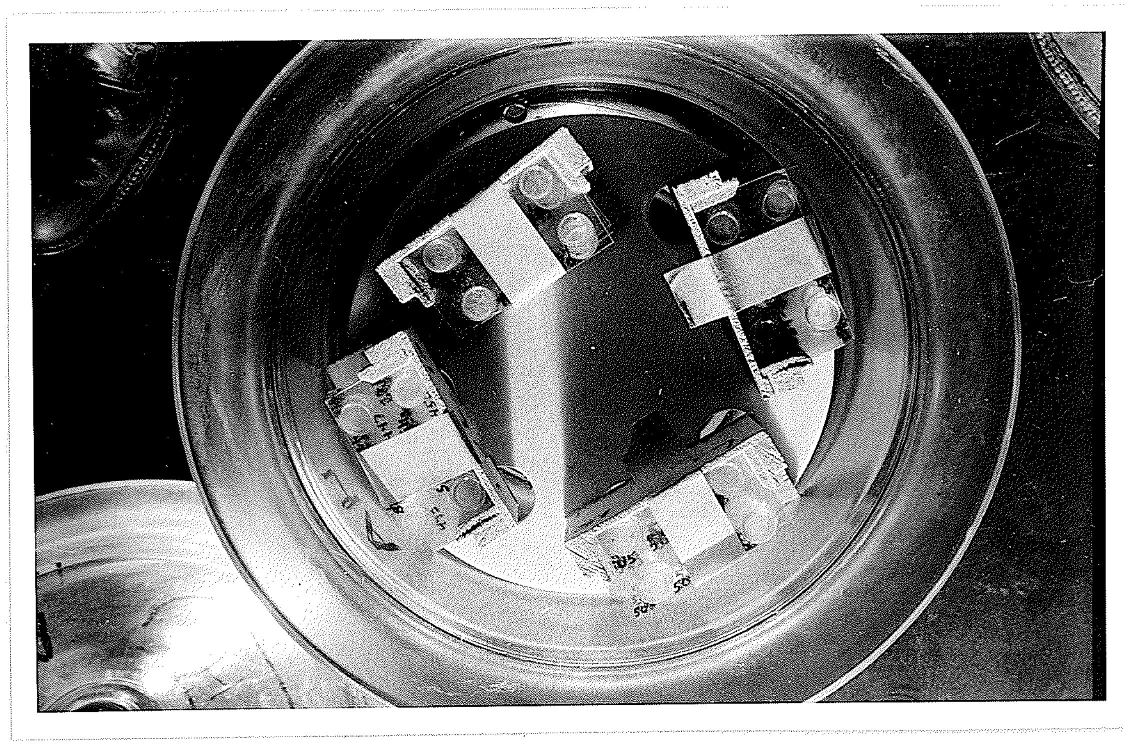

Figure 3. Four lots in a large desiccator.

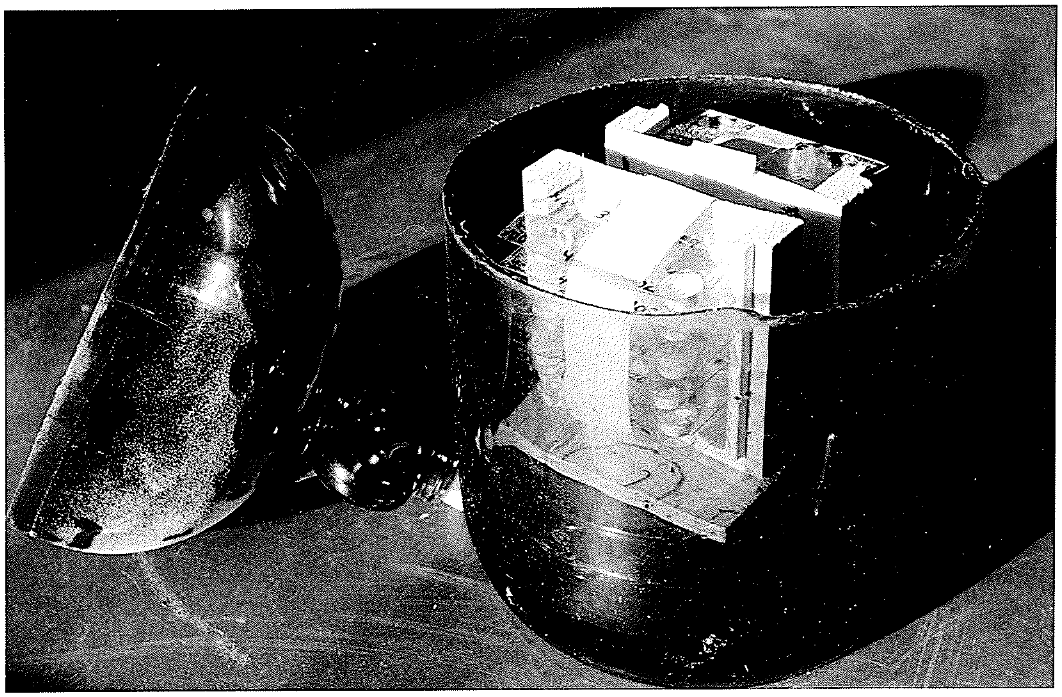

Figure 4. Two lots in a specially constructed desiccator.

tion required for large desiccators was 900 ml.

As fifteen desiccators were required and only nine were available, six had to be constructed. Small mouthed gallon jars were used. These were cut about three-quarters of the way up the jar by means of etching with a glass cutter, then applying a red hot wire under pressure around the etched jar. Stopcock grease was applied and the mouth was corked. As an added protection, masking tape was applied over the two cut edges to ensure the desiccator being airtight. Figure 4 illustrates one of these desiccators.

The foods used for the cultures were whole wheat, bran, ground wheat, and vitamin-enriched flour. These foods were chosen because they represent important stored products which are infested by the hairy spider beetle. For each cubicle 0.231 grams of food were provided. Later, however, to facilitate examination, at least half of the food was removed. More was added later if it was found to be necessary.

The foods were conditioned at various temperatures and relative humidities for at least four days before starting the experiment. Ground wheat was prepared by grinding whole kernels of wheat of 15 per cent moisture content. The particle size of the four foods was measured and this is given in Table VII under the heading, "Weight of Food Fractions Separated by Standard Sieves".

Two criteria for comparing the value of foods were used:

1. Time taken for development of the insect.

2. The weight of the adult beetle reared on a particular food.

When the food was being conditioned to the high temperatures and high relative humidities before the eggs were introduced, mould growth was observed. Hence there was some delay in getting started as the mould had to be removed or food completely changed.

It was intended originally to have thirty-six replicates for each temperature, food, and relative humidity. This would amount to 36 x 4 (foods) x 3 (temperatures) x 3 (relative humidities) or a total of 1,296 eggs. However, towards the end of the experiment there was a limited supply of eggs. Hence in some cases only 18 replicates were used and in the last four lots only 12, 12, 10, and 9 were used. Therefore the actual number of eggs used was 1,051.

The replicates were examined daily or every two days initially. As more eggs were set up it was possible to make examinations only once a week. It was found that larvae could crawl up the walls of the cubicle and escape so precautions were taken to prevent this. The top of each cubicle was covered with a double layer of cellulose tape. Both sticky sides were fused to each other so that the insect would not get stuck to the tape. Holes for breathing purposes were punched through the cellulose tape with a fine needle.

Insects were examined in groups of six under a binocular microscope. Often the egg, or larva, was obscured by food particles and had to be uncovered with a probe. Care was taken not to injure the insect by rough contact. Dates of all significant observations and life cycle changes were recorded for each egg. Usually the cellulose tape covering hindered accurate observation, hence it was temporarily removed at each examination. Food was added when needed, but only among insects with abnormally long periods of development or when diapause occurred. In a few instances it was noticed that with the addition of food larvae pupated within several days.

The mature larva produced a straw-like silky substance. This was spun from the anus. This substance, together with debris and food particles was used to construct a cocoon. After the larva enclosed itself in the cocoon, it stopped feeding. This indicated the approach of pupation. Careful checking was necessary at this stage in order to get accurate dates of change. Soon the larva shed its skin and pupated. This was seen either by direct observation through the underside of the transparent acetate sheeting or by gently lifting the cocoon. The former method was preferred as this was quicker and there was no chance of disturbing the insect.

Towards the end of pupation, movements of what seemed to be the muscles or ligaments or blood were noted under magnification (20x). Soon after, movement of the appendages and mouthparts was noticed. Within two days the adult stage was reached but remained in its pupal case. Again, it was possible to examine the underside of the transparent cubicles to determine when the adult had emerged. After the adult emerged it was immediately weighed.

A Sartorius balance accurate to 0.05 milligrams was used to weigh the adults. The newly emerged adults were carefully brushed to remove food particles or clinging debris. Colour, size, weight, and taxonomic features of the newly emerged adults were recorded. Adults were sexed according to Hinton's taxonomic classification.

Exposure of Adults to Varying Temperatures

Although the study of the effects of constant temperature yields important data relative to development and metabolism it is the variable temperatures which prevail in nature. Hence adults obtained in this experiment were subjected to varying temperatures. It is not known for sure whether this insect has only one generation or two per year. What evidence we do have points to only one generation.

Adults were sexed and put into glass jars, measuring 2 7/8 in. high and 1 3/4 in. wide. A special food medium of ground wheat, plus about 10 per cent vitamin enriched flour, was prepared. To supply the insect with water a wad of wet cotton batting was used. This was fixed to a circle of cellulose tape which in turn was placed on blotting paper. Hence, when the cotton batting was wetted the blotting paper remained dry, insulated by the cellulose tape. In this way the adults could drink water and then dry their mouthparts and legs on the blotting paper before walking on the food. It was found

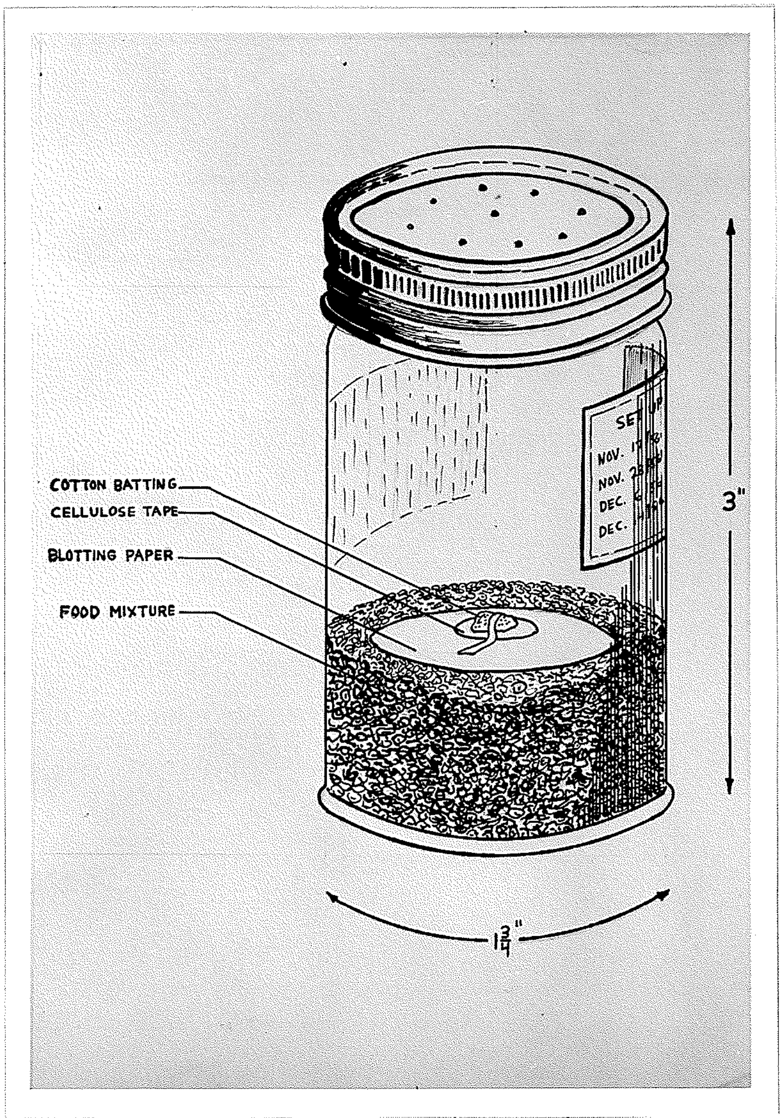

Figure 5. A diagrammatic view of culture container.

Figure 6. Culture containers used.

that if they got their mouthparts and legs wet and then came in contact with particles of food, a paste would form, rendering their mouthparts and legs immobile, and eventually death could result from starvation. Fresh water was given three times a week for the first two months. This was reduced to two times a week, and eventually to just once a week.

Two male and two female adults were placed in each jar. Forty such cultures were set up and subjected to varying temperatures and varying lengths of exposure. Four temperatures were used: 80°F., 68°F., 48°F., and 35°F. Different cultures were exposed for varying lengths of time. The food was periodically sifted for eggs. Very often heavy growths of mould were formed at the higher temperatures. In such cases the medium was sifted for eggs, then discarded. The jar was thoroughly cleaned and replaced with fresh food. Sometimes exceptionally heavy mould growth engulfed the insect, locking its mouthparts and movable joints and eventually caused death. Others less affected, easily survived the mould. Figure 5 shows a diagrammatic view of the container used. Figure 6 is a photograph of several containers used in this experiment with adults.

The statistical methods used are from Snedecor and are given below:

The arithmetic mean was calculated for each set of results. The standard deviation gives a measure of the deviation of each determination from the mean and was calculated using the formula,

$$\sigma = \sqrt{\frac{\epsilon(x - m)^2}{n - 1}}$$ (Snedecor, 1946)

where x is the observed determination, m is the arithmetic mean, n is the total number of determinations.

Analysis of Variance was computed. The results given in Table XIV show the statistical differences between foods, temperatures, and relative humidities in the development of *P. villiger*.

Calculation of the sum of squares and F tests for the four foods are presented in Appendix I, as examples. The other variants were treated similarly.

RESULTS

The Effect of Temperature, Relative Humidity, and Food, on Hatching, Larval Development, and Adult Emergence.

Table I shows that at 80°F. there was a low hatch at 40 per cent relative humidity in all foods except vitamin enriched flour which gave a 52.8 per cent hatch. There was no larval development at this relative humidity. At 60 per cent relative humidity there was a mean hatch of 58.3 per cent and a mean larval development of 67.9 per cent. At 80 per cent relative humidity there was a mean hatch of 65.3 per cent and a mean larval development of 81.6 per cent. Adult emergence was exceptionally high at both 60 and 80 per cent relative humidities.

Results at 68°F. are given in Table II. The hatch at 40 per cent relative humidity is higher at this temperature than at 80°F. Again, vitamin enriched flour appeared to contribute to a higher hatch. No larval development was recorded at 40 per cent relative humidity. There was no significant difference in hatching between insects reared at 60 per cent and 80 per cent relative humidity. Adult emergence at both relative humidities was high but with no significant difference.

Table III shows that at 48°F. there was a low mean hatch at 40 and 60 per cent relative humidities. At 80 per cent relative humidity there was no hatch. No larvae reached the pupal stage at 48°F., although a few were in diapause.

TABLE I

EFFECT OF RELATIVE HUMIDITY AND FOOD ON HATCHING, LARVAL DEVELOPMENT, AND ADULT EMERGENCE AT 80°F.

Relative humidity %	Food	Percentage of insects		
		Hatching	Forming pupae	Emerging as adults
40	Whole wheat	13.89 (36)*	0	0
	Bran	8.33 (36)	0	0
	Ground wheat	19.44 (36)	0	0
	Flour	52.80 (36)	0	0
60	Whole wheat	61.10 (36)	63.6 (14)*	100. (14)*
	Bran	72.20 (36)	69.2 (18)	89. (16)
	Ground wheat	50.0 (36)	61.1 (11)	100. (11)
	Flour	50.0 (36)	77.7 (14)	100. (14)
80	Whole wheat	63.9 (36)	87.0 (20)	100. (20)
	Bran	52.8 (36)	73.6 (14)	85.7 (12)
	Ground wheat	72.2 (36)	88.5 (23)	100. (23)
	Flour	72.2 (36)	72.4 (21)	95.2 (20)

* Number of individuals at start.

TABLE II

EFFECT OF RELATIVE HUMIDITY AND FOOD ON HATCHING, LARVAL DEVELOPMENT, AND ADULT EMERGENCE AT 68°F.

Relative humidity %	Food	Percentage of insects		
		Hatching	Forming pupae	Emerging as adults
40	Whole wheat	22.2 (36)*	0	0
	Bran	30.6 (36)	0	0
	Ground wheat	22.2 (36)	0	0
	Flour	69.4 (36)	0	0
60	Whole wheat	63.9 (36)	88.0 (22)*	74.9 (17)*
	Bran	63.9 (36)	100. (23)	95.7 (22)
	Ground wheat	61.10 (36)	90.9 (20)	100. (20)
	Flour	80.6 (36)	65.5 (19)	100. (19)
80	Whole wheat	63.9 (36)	87.0 (20)	100. (20)
	Bran	69.4 (36)	64.0 (16)	100. (16)
	Ground wheat	63.9 (36)	91.3 (21)	95.2 (20)
	Flour	63.9 (36)	65.5 (19)	66.6 (2)

* Number of individuals at start.

TABLE III

EFFECT OF RELATIVE HUMIDITY AND FOOD ON HATCHING, LARVAL DEVELOPMENT, AND ADULT EMERGENCE AT 48°F.

Relative humidity %	Food	Percentage of insects		
		Hatching	Forming pupae	Emerging as adults
40	Whole wheat	27.7 (18)*	0	0
	Bran	16.7 (18)	0	0
	Ground wheat	11.11 (18)	0	0
	Flour	27.7 (18)	0	0
60	Whole wheat	22.22 (18)	0	0
	Bran	16.7 (18)	0	0
	Ground wheat	11.11 (18)	0	0
	Flour	0. (18)	0	0
80	Whole wheat	0. (12)	0	0
	Bran	0. (12)	0	0
	Ground wheat	0. (10)	0	0
	Flour	0. (9)	0	0

* Number of individuals at start.

TABLE IV

DURATION OF LARVAL AND PUPAL STAGES OF <u>PTINUS VILLIGER</u>
(Number of individuals in brackets)

Temp. °F.	Relative humidity %	Food	1. Larvae, Days	2. Pupae, Days	Total of 1. & 2. (Larvae to adults)
80	60	Whole wheat	(14) 42.14 ± 3.96	(14) 14.00 ± 2.11	(14) 56.14 ± 3.55
		Bran	(18) 39.77 ± 5.04	(18) 13.94 ± 1.87	(16) 53.25 ± 4.08
		Ground wheat	(11) 39.09 ± 1.68	(11) 14.73 ± 2.24	(11) 53.81 ± 2.79
		Flour	(14) 43.27 ± 4.87	(14) 15.33 ± 1.73	(14) 73.78 ± 3.73
80	80	Whole wheat	(20) 42.50 ± 3.62	(20) 14.20 ± 1.43	(20) 56.65 ± 3.86
		Bran	(14) 40.27 ± 4.33	(14) 14.00 ± 1.94	(12) 54.18 ± 4.85
		Ground wheat	(23) 39.83 ± 4.62	(23) 12.87 ± 2.16	(23) 52.21 ± 6.01
		Flour	(21) 47.20 ± 5.25	(21) 14.75 ± 1.32	(20) 60.35 ± 4.79
68	60	Whole wheat	(22) 52.88 ± 6.08	(22) 24.56 ± 4.63	(17) 76.56 ± 5.07
		Bran	(23) 50.14 ± 2.84	(23) 23.78 ± 2.48	(22) 75.04 ± 3.75
		Ground wheat	(22) 55.04 ± 2.02	(20) 22.82 ± 3.27	(20) 76.82 ± 4.25
		Flour	(29) 66.09 ± 3.81	(19) 18.27 ± 1.73	(19) 84.73 ± 3.52
68	80	Whole wheat	(23) 57.37 ± 2.99	(20) 23.42 ± 3.85	(20) 85.45 ± 4.30
		Bran	(25) 53.44 ± 2.98	(16) 21.44 ± 5.48	(16) 75.75 ± 6.08
		Ground wheat	(23) 51.95 ± 2.69	(21) 20.0 ± 3.11	(20) 73.40 ± 3.36
		Flour	(23) 61.00 ±12.73*	(3) 20.5 ± 3.53	(2) 82.50 ± 9.16

*Most were in diapause after 15 days

TABLE V

WEIGHT OF ADULTS AT EMERGENCE FROM COCOONS BRED SINGLY ON VARIOUS FOODS AND TEMPERATURES AT 60% R.H.

Food	Temp., °F.	Number of adults	Weight in milligrams	
			Females	Males
Whole wheat	48	0	0	0
Bran		0	0	0
Ground wheat		0	0	0
Flour		0	0	0
Whole wheat	68	17	2.28 ± 0.212	2.14 ± 0.412
Bran		22	2.51 ± 0.246	2.07 ± 0.249
Ground wheat		20	2.39 ± 0.324	2.13 ± 0.117
Flour		19	2.52 ± 0.355	1.98 ± 0.223
Whole wheat	80	14	3.28 ± 0.184	2.936 ± 0.239
Bran		16	3.33 ± 0.300	2.975 ± 0.388
Ground wheat		11	3.27 ± 0.242	3.22 ± 0.527
Flour		14	2.78 ± 0.257	2.24 ± 0.232

TABLE VI

WEIGHT OF ADULTS AT EMERGENCE FROM COCOONS BRED SINGLY ON VARIOUS FOODS AND TEMPERATURES AT 80% R.H.

Food	Temp., °F.	Number of adults	Weight in milligrams	
			Females	Males
Whole wheat	48	0	0	0
Bran		0	0	0
Ground wheat		0	0	0
Flour		0	0	0
Whole wheat	68	20	3.11 ± 0.514	2.70 ± 0.374
Bran		16	3.04 ± 0.306	2.56 ± 0.232
Ground wheat		20	2.87 ± 0.274	2.38 ± 0.242
Flour		2	3.07 (only 1)	2.63 (only 1)
Whole wheat	80	20	2.96 ± 0.258	2.60 ± 0.256
Bran		12	2.94 ± 0.417	2.34 ± 0.264
Ground wheat		23	3.09 ± 0.432	2.76 ± 0.181
Flour		20	2.82 ± 0.482	2.16 ± 0.368

Duration of Larval, Pupal, and Complete Development of *P. villiger*.

Temperature plays a significant role in its effect on development. The mean number of days required for complete development at 80°F. was 57.55 ± 4.21 days. The mean number of days required for complete development at 68°F. was 78.78 ± 4.93 days.

Duration of development is not significantly different at 60 and 80 per cent relative humidities. A comparison of the four foods with duration of development indicates that development to the adult stage was completed in 64.06 days on ground wheat, 64.55 days on bran, 68.70 days on whole wheat, and 75.34 days on vitamin enriched flour. Figure 15 summarizes duration of development of *P. villiger* and clearly shows these differences.

Weights of Adults at Emergence Reared on Various Foods and Different Relative Humidities at 80°F. and 68°F.

It is evident from these tables that females are heavier than males. The average weight of the female is 2.89 mg., and that of the male, 2.49 mg. The tables show that at 60 per cent relative humidity, average weights of adults were significantly higher at 80°F. than at 68°F. But at 80 per cent relative humidity, average weight of adults was significantly higher at 68°F. than at 80°F. No adults were obtained at 40 per cent relative humidity at either temperature.

Weights of Food Fractions Separated by Standard Sieves

In descending order of coarseness there are ground wheat,

TABLE VII

WEIGHTS OF FOOD FRACTIONS SEPARATED BY STANDARD SIEVES
(10 gm. samples were used for each food)

Food	Size of mesh opening in mm.	Weight of food obtained by mesh in gms.	Percentage of 10 gms.
Bran	1.0	1.52	15.2
	0.75	1.60	16.0
	0.50	3.05	30.5
	through 0.50	3.64	36.4
Ground wheat	1.0	6.57	65.7
	0.75	0.78	7.8
	0.50	1.26	12.6
	through 0.50	0.85	8.5
Whole wheat (kernels)	1.0	0.16	1.6
	0.75	0.34	3.4
	0.50	1.33	13.3
	through 0.50	7.87	78.7
Vitamin enriched flour	1.0	0	0
	0.75	0	0
	0.50	0	0
	through 0.50	10.0	100.

TABLE VIII

EFFECT OF FOOD AND RELATIVE HUMIDITY ON LARVAL MORTALITY OF PTINUS VILLIGER AT 80°F.

Relative humidity %	Food	Number of individuals	Number died	Per cent mortality
40	Whole wheat	36	5	13.88
	Bran	36	3	8.33
	Ground wheat	36	4	11.11
	Flour	36	19	52.77
60	Whole wheat	36	8	22.22
	Bran	36	7	19.44
	Ground wheat	36	8	22.22
	Flour	36	3	8.33
80	Whole wheat	36	2	5.55
	Bran	36	5	13.88
	Ground wheat	36	3	8.33
	Flour	36	4	11.11

TABLE IX

EFFECT OF FOOD AND RELATIVE HUMIDITY ON LARVAL MORTALITY OF *PTINUS VILLIGER* AT 68°F.

Relative humidity %	Food	Number of individuals	Number died	Per cent mortality
40	Whole wheat	36	8	22.22
	Bran	36	11	30.55
	Ground wheat	36	8	22.22
	Flour	36	25	69.44
60	Whole wheat	36	0	0.00
	Bran	36	0	0.00
	Ground wheat	36	2	5.55
	Flour	36	0	0.00
80	Whole wheat	36	1	2.77
	Bran	36	3	8.33
	Ground wheat	36	0	0.00
	Flour	36	0	0.00

TABLE X

EFFECT OF FOOD AND RELATIVE HUMIDITY ON LARVAL MORTALITY OF PTINUS VILLIGER AT 48°F.

Relative humidity %	Food	Number of individuals	Number died	Per cent mortality
40	Whole wheat	18	10	55.55
	Bran	18	3	16.66
	Ground wheat	18	2	11.11
	Flour	18	5	27.77
60	Whole wheat	18	3	16.66
	Bran	18	1	5.55
	Ground wheat	18	2	11.11
	Flour	18	0*	0.00
80	Whole wheat	12	0*	0.00
	Bran	12	0*	0.00
	Ground wheat	10	0*	0.00
	Flour	9	0*	0.00

*Not viable.

TABLE XI

DIAPAUSE OF PTINUS VILLIGER SUBJECTED TO VARIOUS RELATIVE HUMIDITIES AND FOODS AT 80°F.

Relative humidity	Food	Number of individuals	Number completing development	Number in diapause
40	Whole wheat	36	0	0
	Bran	36	0	0
	Ground wheat	36	0	3
	Flour	36	0	2
60	Whole wheat	36	14	0
	Bran	36	16	1
	Ground wheat	36	11	0
	Flour	36	14	0
80	Whole wheat	36	20	0
	Bran	36	12	1
	Ground wheat	36	23	0
	Flour	36	20	3

TABLE XII

DIAPAUSE OF PTINUS VILLIGER SUBJECTED TO VARIOUS RELATIVE HUMIDITIES AND FOODS AT 68°F.

Relative humidity	Food	Number of individuals	Number completing development	Number in diapause
40	Whole wheat	36	0	1
	Bran	36	0	1
	Ground wheat	36	0	1
	Flour	36	0	3
60	Whole wheat	36	17	1
	Bran	36	22	0
	Ground wheat	36	20	1
	Flour	36	19	8
80	Whole wheat	36	20	2
	Bran	36	16	3
	Ground wheat	36	20	1
	Flour	36	2	20

TABLE XIII

DIAPAUSE OF *PTINUS VILLIGER* SUBJECTED TO VARIOUS RELATIVE HUMIDITIES AND FOODS AT 48°F.

Relative humidity	Food	Number of individuals	Number completing development	Number in diapause
40	Whole wheat	18	0	0
	Bran	18	0	0
	Ground wheat	18	0	0
	Flour	18	0	0
60	Whole wheat	18	0	1
	Bran	18	0	2
	Ground wheat	18	0	0
	Flour	18	0	0
80	Whole wheat	12	0	0
	Bran	12	0	1
	Ground wheat	10	0	0
	Flour	9	0	1

TABLE XIV

ANALYSIS OF VARIANCE

Conditions	Degrees of freedom	Sum of squares	Mean square	F
Temperature	1	1803.70	1803.70	134.70**
Humidity	1	5.75	5.75	0.22
Food	3	326.60	108.87	5.50*
[B. & G.W. vs. W.W. & F.[a]	1	237.93	237.93	12.03*
B. vs. G.W.	1	.49	.49	0.02
W.W. vs. F.]	1	88.18	88.18	4.46
Temp. x R.H.	1	19.28	19.28	1.88
Temp. x Food	3	34.28	11.43	1.12
Hum. x Food	3	84.45	28.15	2.75
Temp. x Hum. x Food	3	30.62	10.21	1.12
Total	15	2304.68		

** denotes significance at 1% level.

* denotes significance at 5% level.

a B. = Bran; G.W. = Ground wheat; W.W. = Whole wheat;
F. = Enriched flour.

bran, whole wheat, and vitamin enriched flour. The data show that the coarser ground wheat and bran proved to be a more favourable combination of food than the finer whole wheat and flour.

Effects of Food, Relative Humidity, and Temperature on Larval Mortality.

At 80°F., 68°F., and 40 per cent relative humidity, there was a high mortality rate in vitamin enriched flour. At 48°F. and 40 per cent relative humidity, there was a comparatively high mortality rate in whole wheat. At 68°F. and 60 and 80 per cent relative humidities there was a very low mortality rate in all foods.

Effect of Temperature, Relative Humidity, and Food, on the Incidence of Larval Diapause.

At 80°F. few larvae went into diapause. Table XII shows that at 68°F. more larvae went into diapause in vitamin enriched flour than in any other food. A relative humidity of 80 per cent was most conducive to larval diapause. Table XIII shows that at 48°F. few individuals went into diapause.

Life History and Stages of *Ptinus villiger*.

The post embryonic development of *P. villiger* comprises an orderly series of stages in the course of which the insect becomes transformed from a larva to a pupae and then to an adult. The process involves growth by means of a series of moults. Three larval instars were noted in *P. villiger*. Very little change of form was noted during the larval series of moults. Only increases in size were observed. However, the last larval moult is a striking transformation into the pupal stage. During this pupal stage, the insect does not feed as it continually did in the larval stage, but merely rests, inactive and helpless in an enclosed protective pupal case, the puparium. While in this so-called 'inactive stage' the insect becomes completely transformed into an adult beetle, a marvelous metamorphosis. Figure 7 shows the life stages of *P. villiger*, photographed at about natural size.

The Egg

The eggs are relatively large. When isolated on a black background they can easily be seen with the naked eye. They are about 1/40 of an inch in length and have a diameter of about 1/70 of an inch at the middle. The egg is roughly oval to spindle shaped. When not obscured with adhering flour particles the egg has a pearly opalescence which is usually a good sign of viability. Non-viable eggs usually turn to a dull brown colour. The chorion is moderately translucent, the embryo being dimly visible prior to hatching. The eggs are easily ruptured when fairly young, and have to be handled carefully with a fine camel's hair brush. When the larva hatches, occasionally the egg adheres to it for some time, drawn along with it in search of food. Figure 8 illustrates spider beetle eggs. The rough shape is due to adhering particles of flour.

The Larva

The newly emerged larva is slightly smaller than the

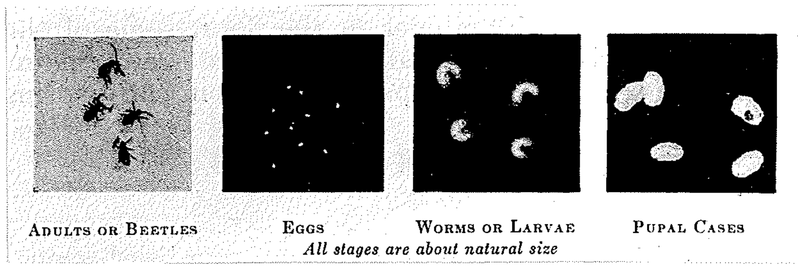

Figure 7. Life stages of P. villiger.

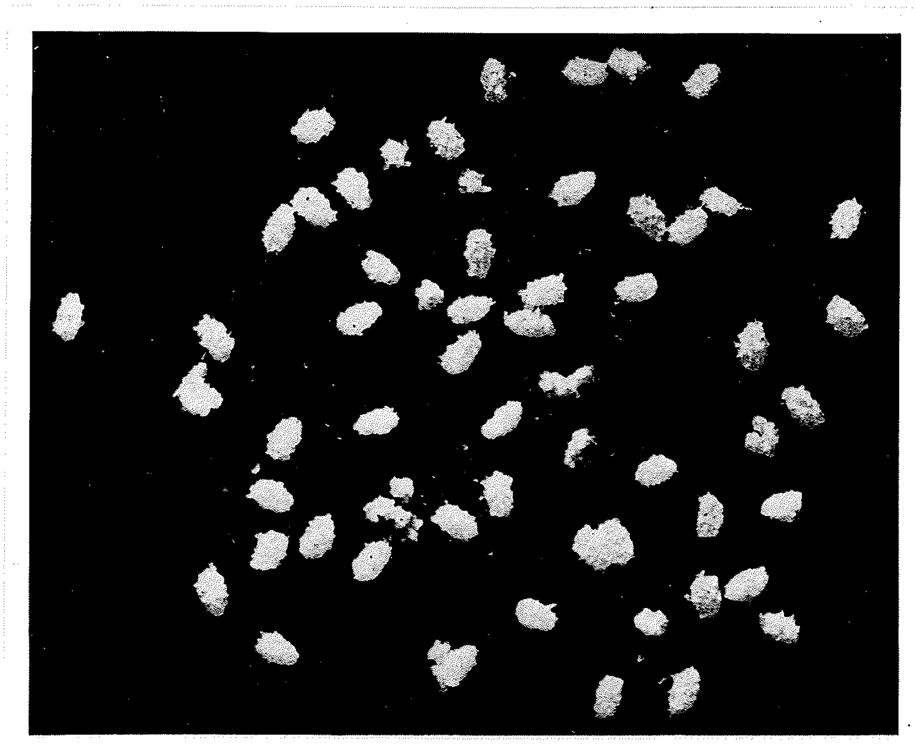

Figure 8. Spider beetle eggs x12.

From Entomology Division, Department of Agriculture, Ottawa.

dimensions of the egg. Fully grown larvae are about 1/6 of an inch in length with a diameter of roughly 1/16 of an inch. They are plump and roundish when well fed, but desiccated, shrunken, and wrinkly when undernourished or thirsting. The body is hairy and generally harbors particles of flour. The larva lies on its side in a curved position when at rest. Generally the color ranges from a light yellow to a creamy white. The head capsule is darker, being a light brown. The mouthparts are dark brown in color.

Before pupation, the mature larva produces a substance which is spun out of the anus in the form of straw-like silk threads. The larva incorporates particles of food, debris and perhaps fecal material to the silky secretion and constructs a cocoon. The cocoon or pupal cell prevents any further feeding and soon the larva sheds its skin and pupation occurs. Figure 9 is a photograph of an original drawing, courtesy Entomology Division, Department of Agriculture, Ottawa. Figure 10 is a photograph of spider beetle larvae, enlarged about 10 times.

The Pupa

The pupal case is usually constructed at the edge of the container so that the two surfaces of the container can be utilized as walls. Figure 11 is a diagrammatic view of the pupal case in a single cubicle. The colour of the pupal case varies with the food medium. Thus in vitamin enriched flour it is white, in bran it is light brown, while in whole wheat and ground wheat it is of a darker shade. The newly formed

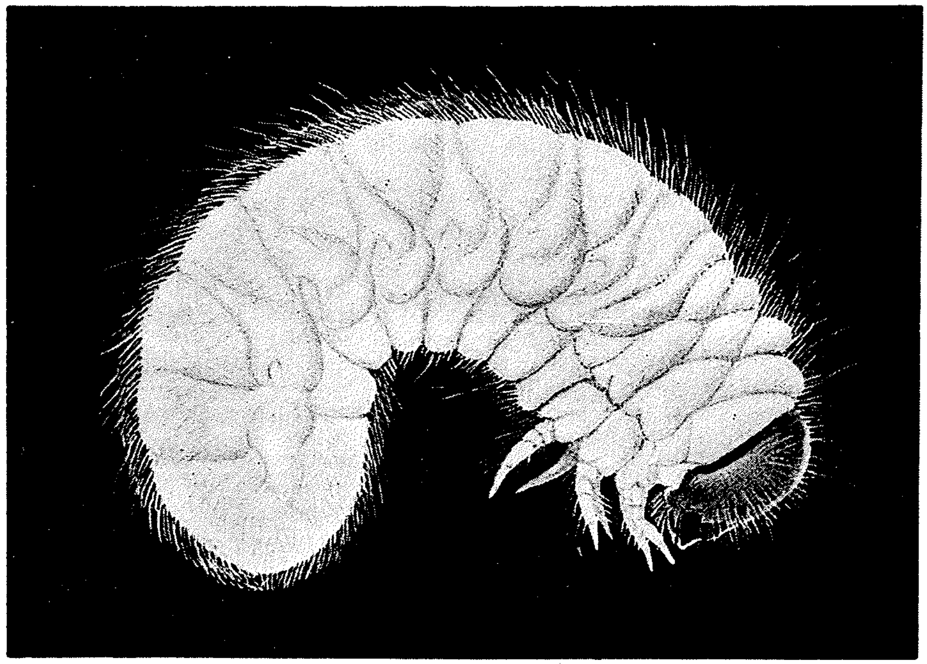

Figure 9. Larva of hairy spider beetle. (Copy of an original drawing, courtesy Entomology Division, Department of Agriculture, Ottawa.)

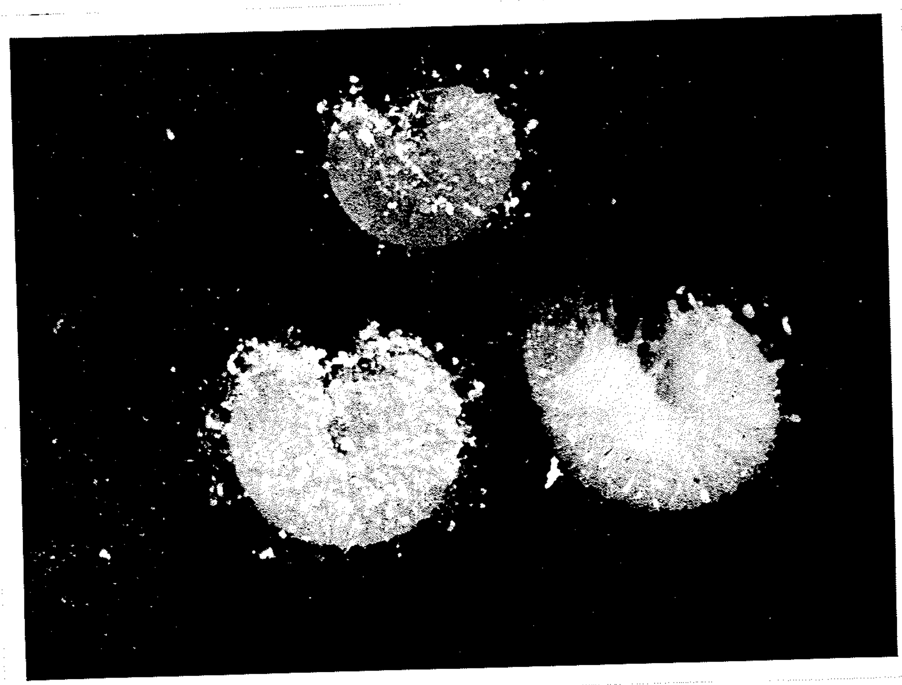

Figure 10. Spider beetle larvae. (Enlarged about x12.)

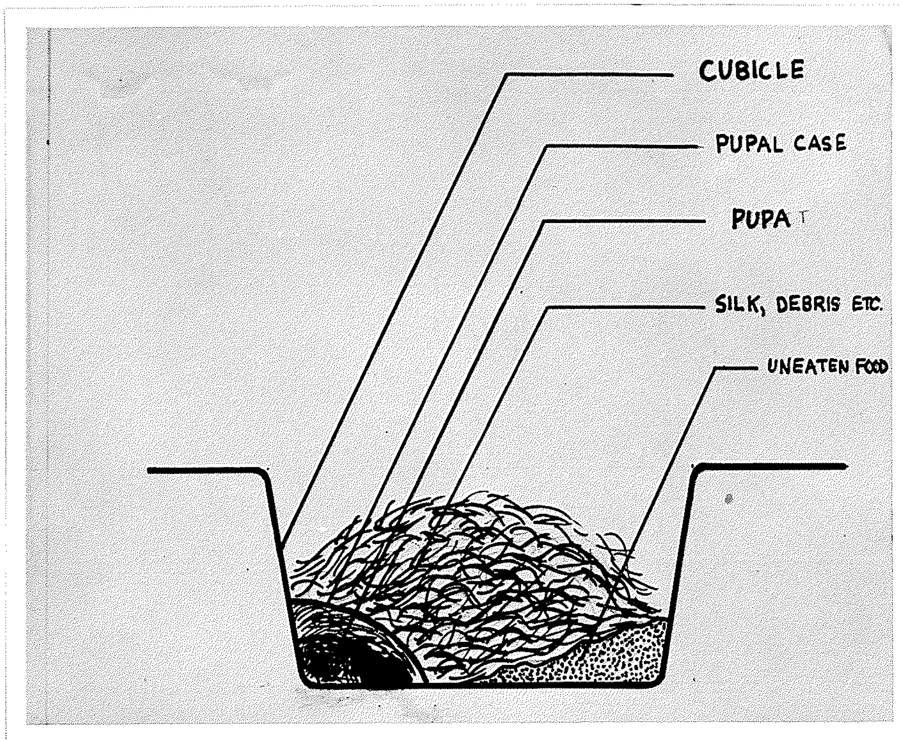

Figure 11. Diagrammatic view of a pupal case in a single cubicle.

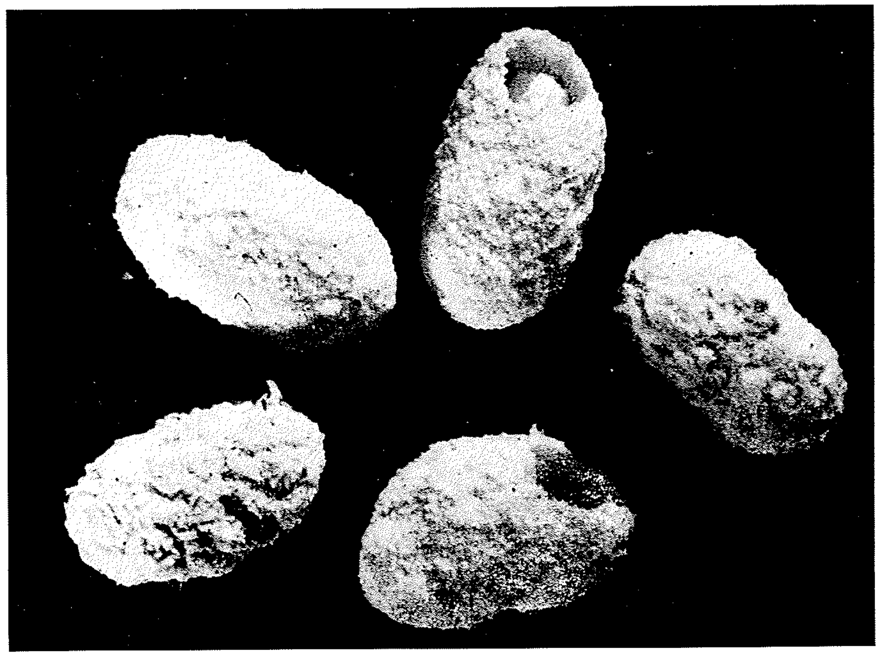

Figure 12. Spider beetle pupal cases x10.

pupa is pearly white. As pupation progresses, it darkens to a reddish-brown.

The wings and legs are free, but enclosed in a sac-like cuticular sheath. Just before the adult is formed, movement of the mouthparts and appendages can be easily seen with a ten power binocular microscope. With a magnification of X20, blood fluid, muscles, or ligaments can be seen moving backwards and forwards. In several cases the actual transformation into an adult beetle was observed, a process which lasted several hours. Immediately after emergence, the adult rested quietly while the cuticle hardened and darkened. Figure 12 shows spider beetle pupal cases.

The Adult Beetle

When the beetle emerged, the entire body was light amber and darkened to a deep brown within a week. Female beetles, upon emergence were always heavier than males. Generally, *Ptinus villiger* is spider-like in form with a dark brown colouration. Usually four irregular light patches are present, two on each elytra. In size the beetles vary from less than 1/8 of an inch to a little more than 1/8 of an inch in length. The female is heavier and has smaller eyes. Its antennae are shorter, with the segments being more or less of uniform length. In the male, the third antennal segment is twice as long as the second one. Usually the male is more slender and less buxom than the heavier female.

The beetles can move about very rapidly but often feign death when disturbed. Blowing warm moist air on the insects usually activates them very quickly. When they are active, the antennae are carried in a widely separated position. Adults retained in cubicles for observation required free drinking water. If this was not given, the insect either bit through the cellulose tape or perished. Figure 13 is a diagrammatic view of the adult beetle after Gibson and Twinn, Bull. 112, Department of Agriculture, Ottawa. Figure 14 is a photograph of adult beetles, enlarged about 15 times.

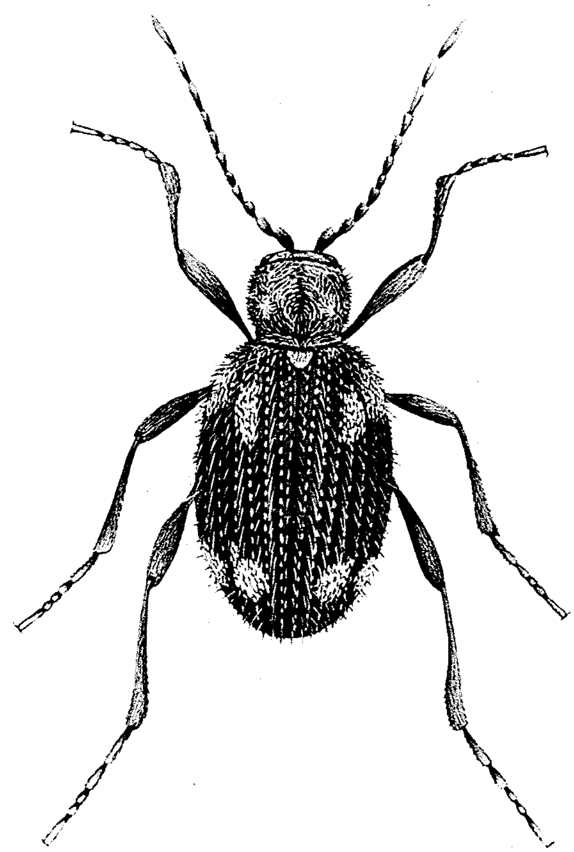

Figure 13. Diagrammatic view of the adult.
(After Gibson and Twinn, Bull.112,
Department of Agriculture, Ottawa.)

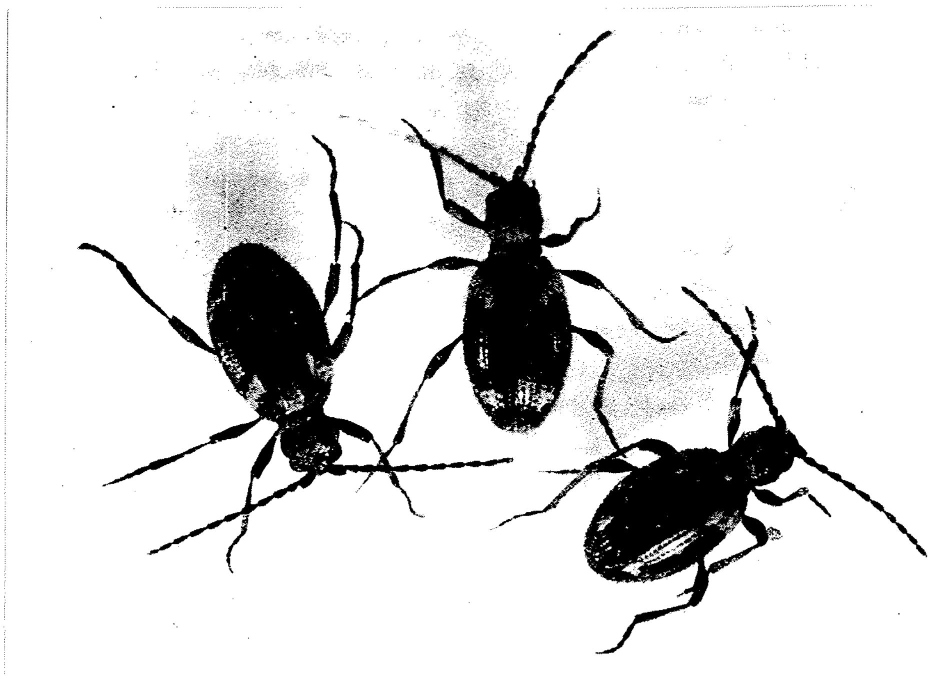

Figure 14. Adult beetles x15.

DISCUSSION OF RESULTS

Rate of development was significantly influenced by temperature ($P < 0.01$). Effects of humidity between 60 and 80 per cent were not significantly different. Forty per cent relative humidity retarded hatching and few individuals reached the larval stage. There was a significant difference between foods on development ($P < 0.05$). It was found that bran and ground wheat were significantly superior to finely ground whole wheat kernels and vitamin enriched flour ($P < 0.05$); the coarser bran and ground wheat favoured faster development. A comparison between bran and ground wheat showed no significant differences. Finely ground whole wheat kernels was more favourable to development than vitamin enriched flour ($P < 0.10$). There was no significant difference among the various interactions of food, temperature and relative humidity.

Effect of Temperature

Reference to Table III and Figure 16 will show that few eggs hatched at 48°F. Most of those that hatched soon died, but a few went into diapause. A true picture of the effect of this temperature on development was not obtained for two reasons. First, fewer replicates were used because there was an insufficient number of eggs near the end of the experiment. Secondly, since few eggs hatched, an inadequate number of larvae were available for measuring the growth rate.

Figure 16, a summary of the hatching aspects of Tables I,

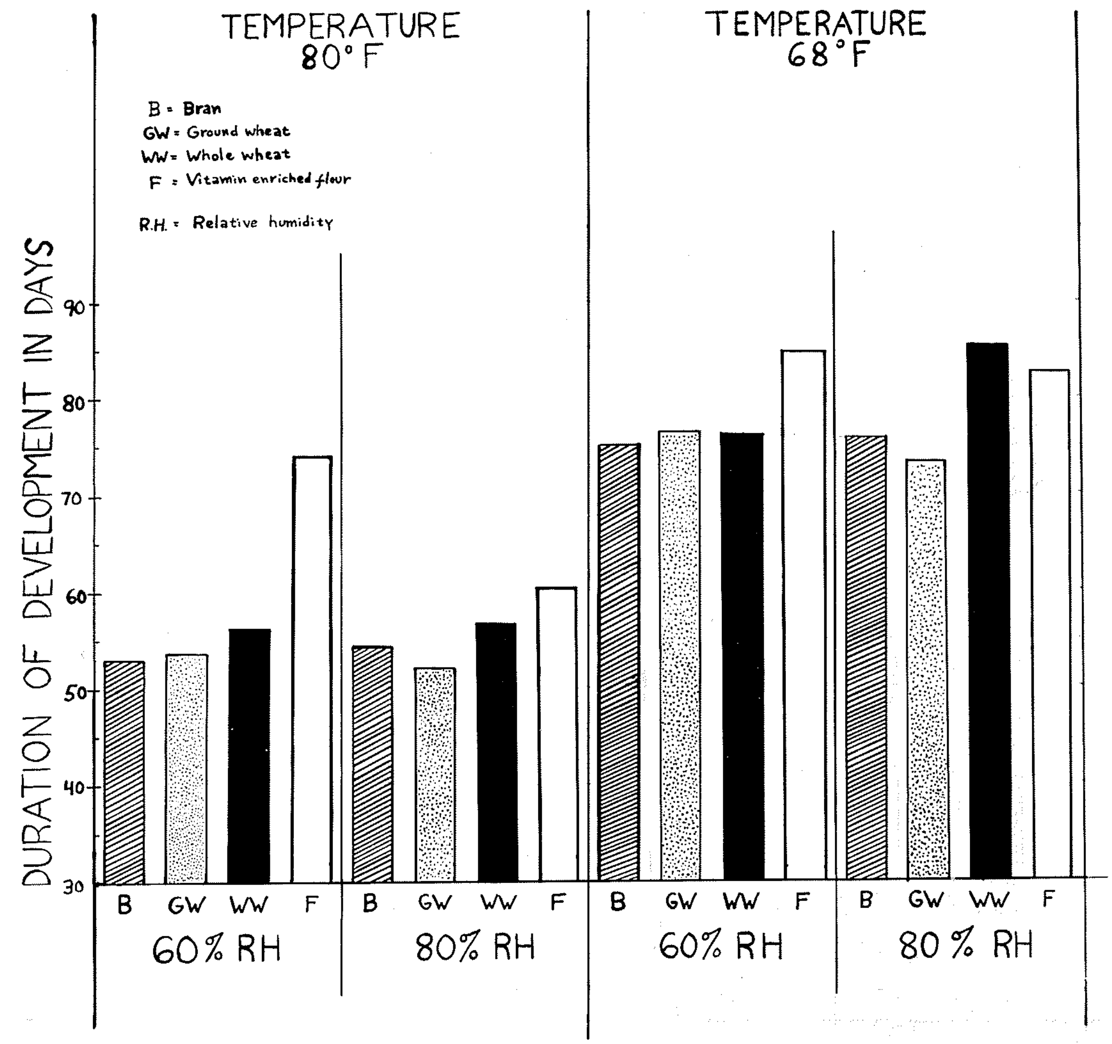

FIG. 15 EFFECT OF TEMPERATURE, RELATIVE HUMIDITY AND FOOD ON THE DEVELOPMENT OF PTINUS VILLIGER

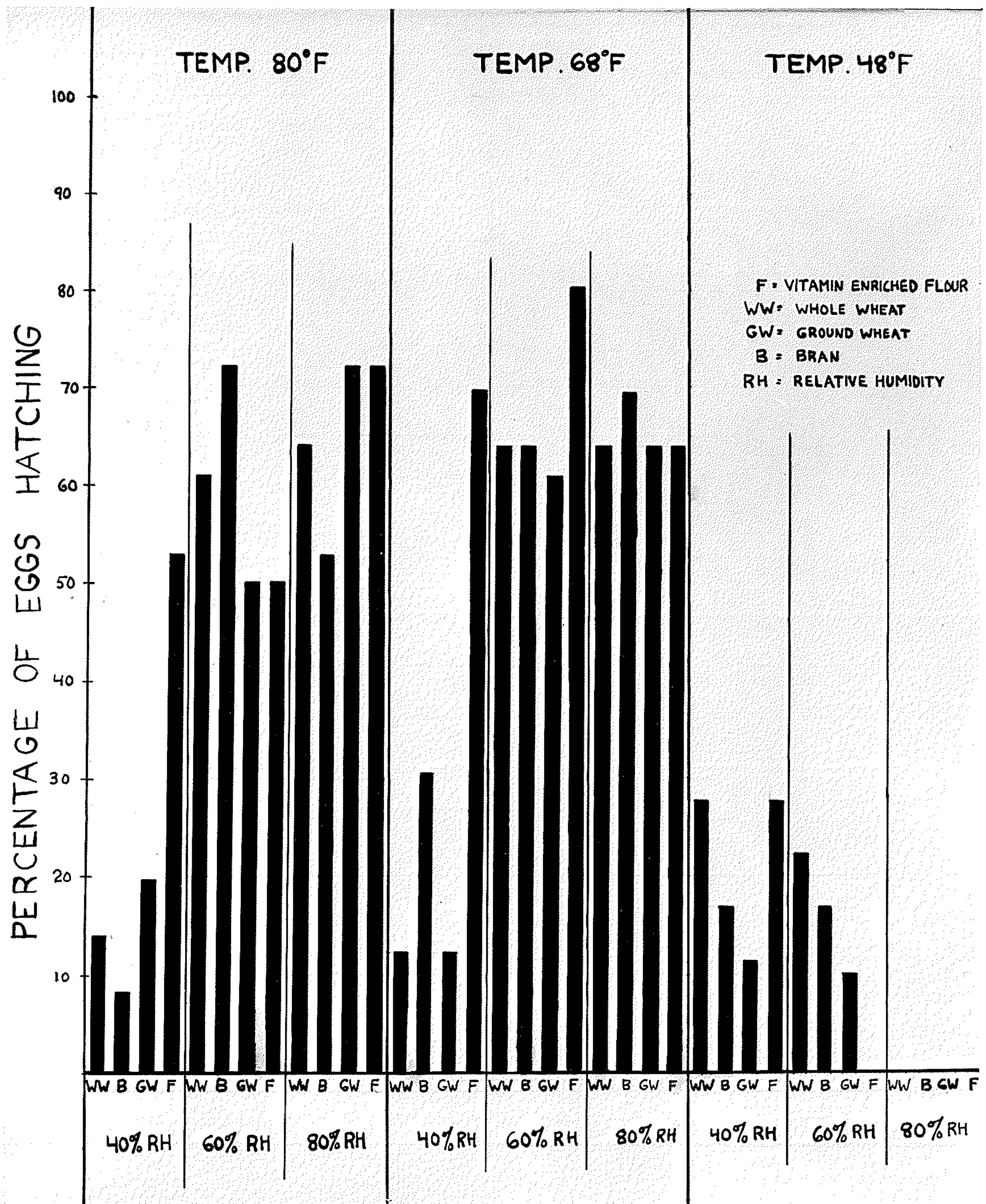

FIG. 16 EFFECT OF TEMPERATURE, RELATIVE HUMIDITY, AND FOOD ON HATCHING

II, and III, shows that optimum hatchability occurred at 80°F. and 68°F. It is apparent that 68°F. was the more favourable of the two. Low relative humidity results in a low hatch even at favourable temperatures (except in vitamin-enriched flour). At 48°F. several live larvae were seen through the chorion of the egg, trapped and unable to extricate themselves. Low hatchability at this temperature suggests that the hatching process (which is largely a matter of muscle power) has a higher temperature coefficient than embryonic development. At 48°F., hatching is low at all humidities and with all foods.

Duration of development of P. villiger is summarized in Figure 15. It can readily be seen that 80°F. favours a more rapid development than 68°F. The average number of days required for complete development at 80°F. was 57.55 ± 4.21 days. The average number of days required for complete development at 68°F. was 78.78 ± 4.93 days.

Effect of Relative Humidity

It is evident from Figure 16 that 40 per cent relative humidity has an interfering effect on the hatching process even at optimum temperatures. When hatching did occur at this low relative humidity it took longer than at the higher humidities. It appears that a combination of a low relative humidity with high temperatures causes desiccation in the egg.

In some cases movement of larvae or mandibles of larvae was detected through the translucent shell, but no development resulted. As with low temperatures, low relative humidity may cause the egg-shell to harden, thus making it more difficult for the larva to chew its way out. Or, on the other hand, perhaps low humidity, having a desiccating effect slows down the activity of the larva, rendering it inadequate to cope with the hatching mechanism.

Only a few accounts have been published regarding the manner in which a young larva extricates itself from the embryonic envelopes. In some cases it is evident that the larva cuts its way out from the egg-shell by means of its mandibles. In others, a specialized organ called a hatching spine has been developed for this purpose. This spine probably ruptures the chorion of the egg and then the young larva proceeds to work its way out (Comstock, 1949). With P. villiger, the larva was observed to use its mandibles in extricating itself from the shell. In no instance was a spine noticed, but this is not evidence enough to conclude that a spine was not used.

The effects of relative humidity on duration of the larval and pupal stages are not significantly different at 60 and 80 per cent. Effects of temperature and food overshadow any slight differences shown here by relative humidity. Prolonged exposure to 40 per cent relative humidity is obviously unfavourable. As well as increasing mortality, the developmental period of the larva is lengthened. Ewer and Ewer (1942), state that larvae of spider beetles eat less at low relative humidities than at high relative humidities.

Effect of Food

The method of rearing individuals in separate cubicles is apparently more satisfactory than if several eggs were introduced into each container. Howe and Burgess (1953), working with Ptinus tectus first used five or ten insects in each container, but results were variable. In general more than one egg in a small container could lead to crowding or cannibalism which would result in an abnormal mortality rate.

Table IV shows that length of larval period was quite consistent when comparing the value of the four foods at various temperatures and relative humidities. The series of foods compared comprises produce normally found in warehouses. The weights of adults at emergence are given in Tables V and VI. However, weight was not used as a criterion in evaluating the effects of the foods on development, as all adults were not weighed at exactly the same time after emergence.

The effect of food on hatching is seen in Figure 16. It appears that on the average, optimum hatching occurred in flour and ground wheat. However, rate of development was significantly slowest in flour and finely ground whole wheat.

The particle size of these foods affected the feeding habits of P. villiger contrary to what was expected. The finer foods produced the slowest development whereas the coarser foods caused most rapid development. There was a correlation between particle size and rate of development; ground wheat, the coarsest of foods produced the most rapid development, taking 64.06 days;

bran, the second coarsest food took 64.55 days, finely ground whole wheat took 68.7 days, and vitamin enriched flour, the finest of the four foods took 75.34 days.

It would appear that particle size, or texture of the food seems to be tied up with the formation of the cocoon. The flour and finely ground whole wheat, being of fine texture, perhaps, required a longer time to form into the silky, straw-like substance secreted by the larva prior to formation of the cocoon. The coarser food substance could have contributed to a more rapid formation of the cocoon, and hence a shorter larval period. This consequently would have resulted in a shorter total developmental period. Also, the fact that the cocoon is comprised of particles of food, debris, and silk supports this view. Naturally, the larger the particle of food, the quicker it will form a cocoon covering.

Vitamin enriched flour appears to be the most inconsistent of the four foods. In it the highest percentage of hatching occurred, the longest developmental period resulted, and the highest incidence of larval diapause was found.

Incidence of Diapause

Reference to Figure 17 shows that few individuals went into diapause at 48°F. and 80°F. At 68°F., more larvae went into diapause in vitamin enriched flour than in any other food. Moreover, there was a progressive increase in diapause as relative humidity increased (in flour).

It is a known fact that growth, as well as reproduction may

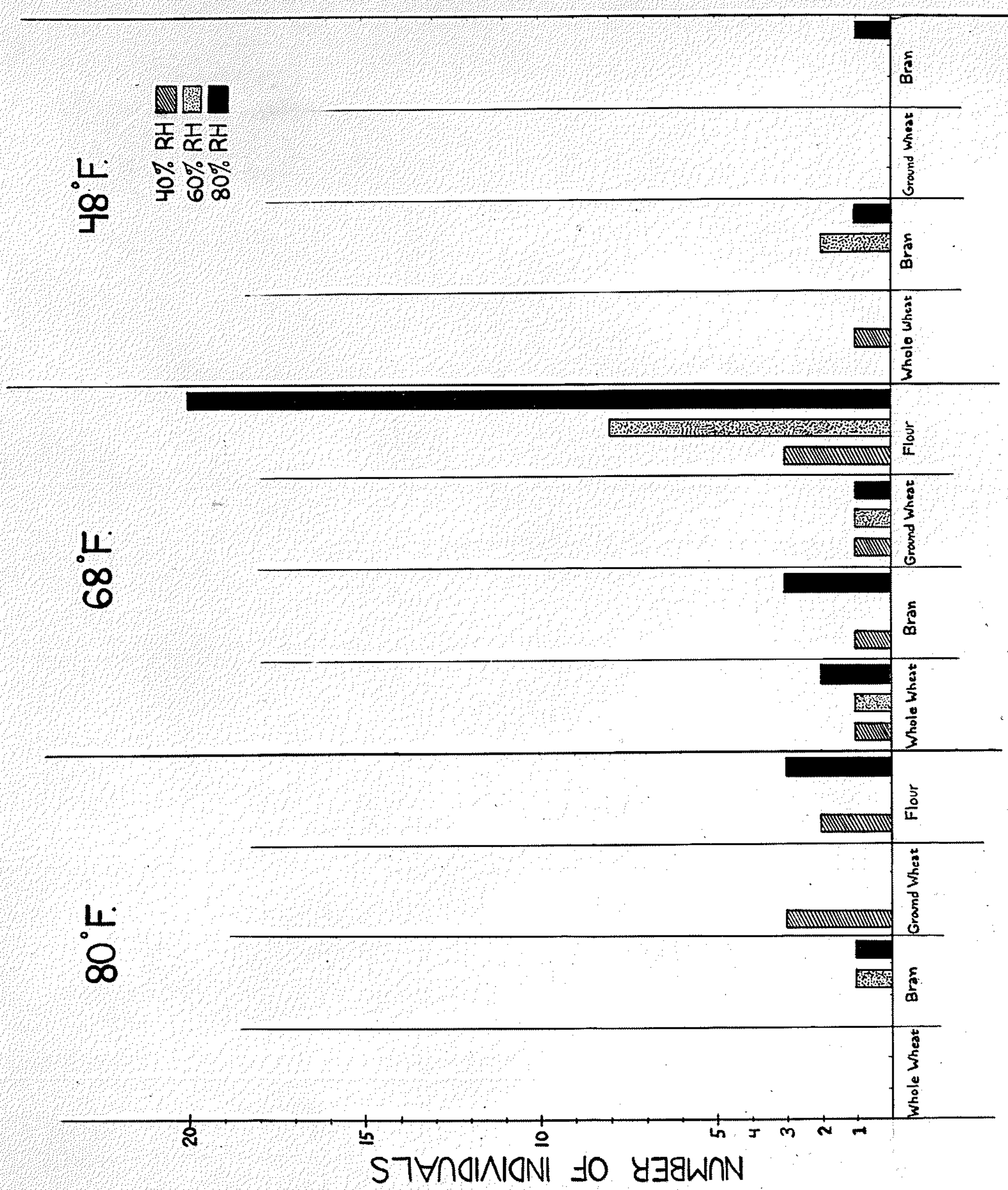

FIG.17 INCIDENCE OF DIAPAUSE

suffer a periodic arrest. With insects there are two types of diapause. First there is the group in which a continuous succession of generations occurs so long as conditions are favourable and in which growth is arrested only by the direct action of adverse circumstances, such as cold, drought, improper diet, or starvation. This group is termed 'homodynamic'. Second there are those insects which show at some stage of their life history a prolonged arrest of growth which supervenes irrespective of the environment. These are termed 'heterodynamic'.

P. villiger has illustrated a homodynamic type of diapause. Those insects reared at 80°F. and low relative humidity, which survived desiccation, became shrivelled, dwarfed and failed to develop beyond the larval stage. Several third-instar larvae, large and healthy looking, went into diapause if additional food was not given after their original supply was used up. At 68°F., twenty larvae went into diapause in vitamin enriched flour at 80 per cent relative humidity. It appears that this combination of food, temperature, and relative humidity produces a certain inhibitory stimulus affecting further larval development.

It is evident from Tables V and VI that females are heavier than males. The average weight of the female is 2.89 mg. and that of the male 2.49 mg. Body weight is distinctly heavier just after emerging. Weight is progressively lost as time passes. This is probably due to a certain amount of desiccation after emergence. Also increased activity of the adult may result in a loss of energy and a consequent loss in weight.

The total number of females obtained was greater than the total number of males. This is of interest in light of Watters' (1954) finding a greater incidence of females on bags of stored products in warehouses. Also, he found more dead females on the sprayed floors of warehouses than males. The total percentage of adult females emerging was 57%. The total number of adult males emerging was 43%.

SUMMARY

1. An evaluation of the effect of food, temperature, and relative humidity on the life cycle of *Ptinus villiger* (Reit.) was made. Results show that rate of hatching was influenced by temperature. Few eggs hatched at 48°F. At 68°F., 56.25 per cent of the eggs hatched. At 80°F., 41.56 per cent of the eggs hatched.

2. Duration of development was significantly influenced by temperature. Results show that 80°F. favours a more rapid development than 68°F. The average number of days required for complete development at 80°F. was 57.55 ± 4.21 days. The average number of days required for complete development at 68°F. was 78.78 ± 4.93 days.

3. Results show that 40 per cent relative humidity has an interfering effect on the hatching process, even at optimum temperatures. It is thought that the combination of low humidity with high temperature resulted in desiccation of the egg. At 60 and 80 per cent relative humidity no significant differences were noted on duration of larval and pupal stages. The effects of temperature and food overshadow any slight differences shown by relative humidity.

4. Insects bred on ground wheat took 64.06 days to develop completely. Bran resulted in 64.55 days, finely ground whole wheat in 68.70 days, and vitamin enriched flour took 75.34

days. It was found that the coarser foods, ground wheat and bran were superior for development than the finer whole wheat and flour.

5. There was no significant difference among the various interactions of food, temperature, and relative humidity.

6. Average weight of females upon emergence was found to be 2.89 mg., that of males 2.49 mg. Body weight was distinctly higher just after emergence and weight was progressively lost as time passed. It is thought that this loss of weight is due either to desiccation after emergence or to a loss of energy due to increased activity.

7. Few larvae went into diapause at 48°F. and 80°F. At 68°F., more larvae went into diapause in vitamin enriched flour than in any other food. A relative humidity of 80 per cent was most conducive to larval diapause in vitamin enriched flour.

8. Adults subjected to varying temperatures of 35°F., 48°F., 68°F., and 80°F. were found to require free drinking water. If this was denied them, they perished. Through-out the winter these insects received an adequate supply of food and water. No eggs were laid during the winter and early spring.

9. The hairy spider beetle larvae will thrive at warm temperatures of 68°F. and 80°F. and high relative humidities. They

prefer coarser ground wheat and bran to a more fine food such as vitamin enriched flour and finely ground whole wheat.

APPENDIX I

Calculation of the sum of squares and F tests are presented here. Other variants are treated similarly.

Food ss =

$$\frac{\overset{\text{(Whole wheat)}}{(56.14 + 56.65 + 76.56 + 85.45)^2} + \overset{\text{(Bran)}}{(53.25 + 54.18 + 75.04 + 75.75)^2} + \overset{\text{(Ground wheat)}}{(53.81 + 52.21 + 76.82 + 73.40)^2} + \overset{\text{(Flour)}}{(73.78 + 60.35 + 84.73 + 82.50)^2}}{4}$$

$$- \text{ correction term} = \frac{(56.14 + 53.25 + 53.81 \; \ldots\ldots \; 82.50)^2}{16} =$$

74,667.35 - 74,340.75

= <u>326.6</u>

<u>Next the interaction of the foods was calculated</u>:

1. (bran and ground wheat) vs. (whole wheat and flour) ss =

$$\frac{(258.22 + 256.24 - 274.8 - 301.36)^2}{16} = \underline{237.93}$$

2. (bran) vs. (ground wheat) ss = $\frac{(258.22 - 256.24)^2}{8}$

$$= \underline{0.49}$$

3. (whole wheat) vs. (flour) ss = $\frac{(274.8 - 301.36)^2}{8} = \underline{88.18}$

(The three totals = 326.6 which is equal to the food ss)

Next the mean square was calculated:

M.Sq. = ss ÷ degrees of freedom (which is 3 for the four foods)

$$= \frac{326.6}{3} = 108.88$$

Finally the F test was made:

$$F_{3,6} = \frac{\text{M.Sq. for food}}{\frac{\text{ss for temp.x hum.} + \text{ss for H x F}}{6}} = \frac{108.88}{\frac{118.73}{6}} = 5.50$$

F 3,6 is F test with the appropriate degrees of freedom.

3 = degrees freedom for food.

6 = degrees freedom for temp. x humidity combination. plus D.F. for humidity x food combination.

Therefore, F 3,6 referred to in Tables is 4.76 for 5% level

and 9.78 for 1% level,

since our answer is 5.50 or above 4.76 and below 9.78.

Food is significant at the 5% level but not at the 1% level.

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