Artificial kernels and food bands for raising Sitophilus oryzae (L.) on microscopic concave slides

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Summary. Artificial kernels and food bands (1 mm thick) stretched on concave microscopic slides proved inviting and very suitable for oviposition and for completing the life cycle of Sitophilus oryzae (L.). This artificial medium contains jelly*, wheat flour and corn oil, its moisture content is 18.1%. This is the first time it was possible to breed an insect on artificial media on glass slides.

It is the aim of this work to bring the immature stages of Sitophilus Oryzae to the spotlight and expose it to the investigator's eyes. Firstly, the weevils are fooled into ovipositing in artificial soft wheat-like kernels which can be completely dissolved in water to obtain the eggs sound and intact. Secondly, these kernels are pressed against concave slides 8×3.5 cm to form food bands on which the larvae lived. Many workers dissected the kernels in an attempt to study the immature stages of this insect and consequently killed the specimens[2–4]. The latter authors followed this insect from egg to adult emergence by using the X-ray technique developed by Milner et al.[5]. Chippendale[6,7] used refined diets of casein, yeast, corn starch, etc., compacted into gelatine capsules or formed in pellets to feed Sitotroga cerealella.

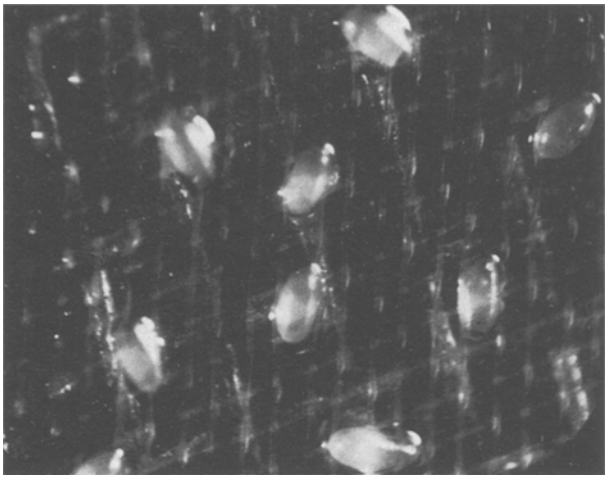

Fig. 1. Eggs of S. oryza separated from artificial kernels on a screen (1600 holes/cm^2).

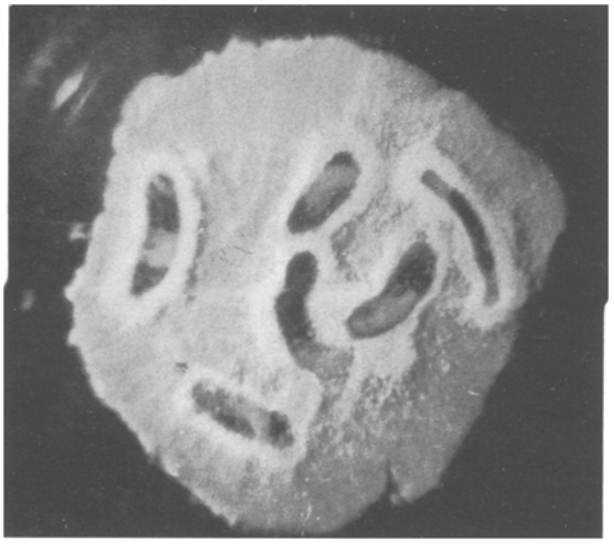

Fig. 2. Larvae and pupae of Sitophilus oryzae growing on artificial medium in a depression of a concave slide.

Materials and methods. Identification and sexing. S. oryzae was distinguished from S. zeamais by the characters (which are 98–99.7% reliable) given by different authors[8–12]. They differ in life cycle[13], response to light and temperature[14], the plasmids they contain[15] and lipid contents[16]. Adults of S. oryzae were sexed according to Halstead[10] by the direction of the 5th and 6th abdominal sternites and the dimensions of the rostra which were measured by ocular micrometer in 15 males and females and averaged.

Artificial kernels. These kernels were made of 25 g of Jelly* crystals in 70 ml of boiling water; after refrigeration they were mixed with wheat flour (5:6 by wt). Pure corn oil was added at concentration of 1%; the dough was kneaded and formed into kernels (without crease), air-dried for 24 h and kept in the refrigerator. 10 pairs of weevils (3 weeks old) were kept in 3×4. 5 cm plastic containers and covered with filter paper glued caps for aeration and were provided with ten artificial kernels with or without 3 g of wheat bran. The suitable grain and air moisture and temperature for oviposition of S. oryzae were taken into consideration[13,17,18]. Artificial kernels contain 18.1% water and were assessed for oviposition at 25 $\pm$ 1 °C and 70% RH for one generation F_2, and at room conditions (26.9 °C and 70.3% RH) for the other F_1. The kernels were daily renewed and the replaced kernels were used for egg counts in 2 ways: 1. They were kept in bottles for 32–42 days for counting the emerging adults. Hindi wheat kernels were used in this experiment for comparison. 2. The artificial kernels were dissolved in

* Jelly crystals, made by St. George's Factory, Alexandria (ARE), contain: sugar, gelatin, citric acid and artificial flavor.

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Table 1. Eggs deposited on artificial and natural (Hindi) wheat grains offered to Sitophilus oryzae (L.) at the rate of one grain/one pair of insects. Eggs were counted by the 2 tabulated methods.

Methods of counting eggs	Generation	Eggs/5 artificial kernels/day with bran	Eggs/5 artificial kernels/day without bran	Eggs/5 natural kernels/day
1 Dissolved artificial kernels	*F_1	3.9 ± 8.5	3.25 ± 4.9	-
	*F_1	3.24 ± 2.4	2.83 ± 1.18	-
2 Number of emerging adults 32–42 days later	*F_1	0.32	0.38	0.24
	*F_2	0.30	0.24	0.29

* F_1 = Laboratory conditions 26.9 °C and 70.5% RH; F_2 = 25 ± 1 °C and 70% RH.

water 35 °C for 2 h in a cylindrical tube provided with a screen (mesh 1600 cm^2). Then eggs were separated from gelatinous debris of kernels by running water (figure 1). In these 2 experiments, the weevils used belong to 2 successive generations kept entirely on artificial kernels. The first generation was kept at laboratory conditions (September, October 1974 average temperature 26.9 °C, 70.5% RH), whereas the second was kept at 25 ± 1 °C and 70% RH (January to February 1975). Kernels were pressed against the concave slide in bands about 1mm thick (figure 2). Eggs or first instar larvae were placed on the surface or in holes made by a needle in the band. The slide was covered by a plane or another concave slide according to the size of the growing larvae. The concave slides were kept in a dessicator at 25 ± 1 °C and 80% RH (10.36% H_2SO_4). 150 first instar larvae were used to determine the 4 larval instars and the pupal period by 3 ways: a) measuring head capsule width by ocular micrometer, b) marking larvae with car paint to fix the moulting date in the first 2 instars and observing the cast head capsules in the later instars, c) measuring tunnel width with ocular micrometer.

Results and discussion. The variables which make the artificial kernels acceptable were controlled in these experiments as texture and size of kernels[13], stability of the kernel during oviposition[19], density of weevils in relation to the number of grains[18] and the type of flour favoured by the weevils[8, 14]. The addition of gelatin conferred softness and stickiness to the artificial kernels (which helped in the stability of kernels during oviposition), also the addition of bran to the tubes containing the artificial kernels.

Oviposition. By searching 2 groups each containing artificial kernels given to weevils with or without bran at room conditions (F_1) the average numbers of eggs/kernel day were 3.9 and 3.25 respectively under controlled conditions (F_2) 25 ± 1 °C and 70% RH, the corresponding averages were 3.24 and 2.83. The variations expressed as standard deviations in egg laying were greater in F_1 than in F_2 ± 2.4 and 1.18 with and without bran. These differences proved to be significant at the 0.05 level.

Oviposition and survival of S. oryzae on wheat and artificial kernels with and without bran were studied by counting the emerging adults in 150 kernels under laboratory conditions and in 75 kernels under controlled conditions. The averages of emerging adults were respectively 0.32 and 0.38 per artificial kernel with and without bran and 0.24 per natural kernel in respect in F_1. Whereas the corresponding figures for F_2 were 0.3, 0.24 and 0.29 adults per kernel. The addition of bran increased oviposition when assessed by dissolving the kernels and counting the deposited eggs (table 1) in both F_1 and F_2. The added bran serves as excess food for the weevils and helps in the stabilization of the kernels during oviposition. The results were not as decisive when the surviving or emerging adults were counted. The natural mortality of hatching larvae within the kernels is apparently high.

Incubation period and hatching. A total of 285 eggs freed from artificial kernels were incubated at 25 ± 1 °C and 100% RH in petri dishes. Hatching occurred from 4 to 7 days with an average of 5.3 days and of hatchability varied from 65 to 86.3% with an average of 77.2%.

Pre-oviposition period. 15 pairs of newly emerged weevils from natural and artificial kernels were provided each with 2 artificial kernels/pair, the kernels were daily changed. Eggs were counted in replaced kernels for 11 days to determine the preoviposition period and the number of eggs daily laid for 1 week thereafter. At 25 °C and 70% RH, the average period was nearly the same (3.6 days) for adults emerging from natural and from artificial kernels. Females emerging from artificial kernels laid 39.5 eggs per 7 days in comparison with 37.7 eggs for females emerging from natural kernels. These differences were insignificant. This again supports the view that

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Table 2. Mean duration of larval and pupal periods of S. oryzae (L.) in days at 25 ± 1 °C and 80% RH on artificial food bands in concave slides and percent mortality of unmarked (–) and marked instars (+).

	Larval instars 1st	2nd	3rd	4th	Prepupa	Pupa	Total
	– 5.8	5.6	5.8	6.2	1	6.5	30.9
Mean	+6.9	6.1	5.9	6.04	1	6.3	32.24
Range	– 5–7	5–7	5–7	6–7	1	5–8	27–37
	+5–8	4–7	4–7	4–8	1	4–8	22–39
Percent mortality	– 50	20	10	6	6	0	
	+47	25	22.5	13	7.5	0	

Table 3. Tunnel length and ratio of tunnel width to head capsule width in the 4 larval instars

	1st instar	2nd	3rd	4th
Tunnel length	4.0 mm	7.0	8.0	12
Tunnel width (TW)	0.26 mm	0.36	0.62	1.06
Head capsule width (HW)	0.19 mm	0.24	0.32	0.44
TW/HW	1.40	1.47	1.9	2.4

artificial kernels are as successful as the natural ones in feeding S. oryzae. No parthenogenesis is practiced by S. oryzae and virgin females do not lay any egg[20]. Nonetheless no failure in egg laying was encountered in any of the 30 pairs, which proves that the artificial kernels were as successful in producing efficient males as the natural ones.

Larval and pupal periods. 100 newly hatched larvae were marked with car paint and put in small holes made in food bands with 50 unmarked ones to study their duration. Results are indicated in table 2. Although temperature and RH were different in the present work, there is almost complete agreement between the figures tabulated and those given by Sharifi and Mills[4]. The tabulated figures also show that marking the larvae by paint may increase mortality, which, however, was high in the first instar whether marked by paint or not. The high percentage (70.2%) of mortality in larvae hatching on surface of food bands is attributed to inability of the hatching larvae to bite into the band surface; larvae hatching from eggs placed in holes in the food bands could bite into the walls of their holes. Their mortality percentages were far less (37.5%) than those of transferred first instar larvae (47–50%); this may indicate that normally mortality in the first instar is not as high as indicated in these experiments (47–50%). The occurrence of molting in the different instars was recognized by the absence of the marks from molted larvae and the presence of cast head capsules. Head capsules and tunnel widths of 10 larvae each of the 4 instars were measured (table 3). The ratio of tunnel width to width of the head capsule is not constant, as it increases slightly in the first 2 instars and abruptly in the third and the fourth. This may serve the future requirement of the next stages or it allows the fourth instar to turn head to tail in its tunnel.

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Progressive impairment in high energy phosphate pattern induced by intermittent coronary perfusion

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Summary. After continuous myocardial ischemia, ATP and CP are both depressed. A different pattern is exhibited in the course of intermittent coronary perfusion. The drop in ATP stores is not avoided, but progressive rise in CP contents is observed.

Ischemia has repeatedly been demonstrated to produce dramatic changes in myocardial function, cell structure and metabolism. Some data support the fact that short adequate coronary perfusion, associated with periods of ischemic arrest, could offer the advantage both of easy operative conditions and of reduced myocardial injury when compared to continuous aortic cross-clamping[2,3]. Such a concept may at first appear valuable. Coronary perfusion supplies substrates, namely glucose, and eliminates acidic components which may potentiate the deleterious effects of ischemia, particularly the loss of integrity of mitochondria[4]. Conversely reoxygenation has been shown to enhance some damage caused by ischemia, such as disruption of plasma membrane associated with enzyme release[5].

One of the effects of ischemia is the impaired ability to generate energy, a fact which is demonstrated by the decrease of high energy phosphate levels in the post-ischemic period. For Sakai[6], the more the myocardial ATP is decreased the more the rate of enzyme release is increased. In this study, the effect of intermittent coronary perfusion on the levels of ATP and CP during a series of ischemic and reperfusion periods was examined. A gradually increasing accumulation of CP was demonstrated.

Material and methods. 7 mongrel dogs, weighing 20–25 kg, were studied. Each dog was anaesthetized with pentobarbital (20 mg/kg). After tracheal intubation, ventilation was achieved with a volume controlled respirator. The animals underwent right thoracotomy through the fourth intercostal region. Heparin (3 mg/kg) was administered and the animals were submitted to extracorporeal heart-lung bypass. A bubble oxygenator with a heat exchanger primed with Ringer in order to reduce the hematocrit around 25% was used. The temperature was maintained at 37 °C and arterial pressure, pH, pO_2 and pCO_2 were monitored during the whole procedure. The heart was exposed in order to have an easy approach to the left ventricle avoiding any further handling of the heart. The aorta was cross-clamped for 15 min and then, by releasing the clamp, reperfusion was initiated for 3 min at a pressure of 80 mm Hg. During reperfusion, ineffective and irregular, heart beats could be

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