AN UNUSUAL MORTALITY OF *SITOPHILUS ZEAMAIS* MOTS. CAUSED BY *SITOTROGA CEREALELLA* (OLIV.) IN MIXED LABORATORY CULTURES

J. N. AYERTEY
Department of Zoology
University of Cape Coast
GHANA

INTRODUCTION: Laboratory observations made on a series of mixed species cultures of the maize weevil, *Sitophilus zeamais* Motschulsky and the Angoumois grain moth *Sitotroga cerealella* (Olivier) cultured on maize showed that *S. zeamais* eliminated *S. cerealella* from all the mixed cultures. However *S. zeamais* suffered a high initial mortality which affected its initial growth in all the mixed cultures (Figure 1). In the 30 experimental jars in which the two species were cultured together, the mean mortality of *S. zeamais* recorded 30 days after the beginning of the experiment amounted to 48%. The mortality of *S. zeamais* cultured alone in similar jars amounted to only 7% during the same period. This difference was surprisingly high ($P < .01$) and a series of investigations were carried out to determine the cause.

EXPERIMENTAL: Source of experimental insects: *S. zeamais* and *S. cerealella* used in these experiments were from stocks originally maintained on wheat for about 10 yr at the Pest Infestation Control Laboratory in Slough, U.K. These were subsequently subcultured on maize for about 2 yr in the same laboratory where this work was carried out. The maize was imported from Ghana and as far as could be ascertained had not been treated chemically. All the cultures and experiments were maintained in a Constant Temperature and Humidity room at 30°C and 70% r.h.

Stage of *S. cerealella* causing mortality of *S. zeamais*: In the culture jars in which the unusual mortality of *S. zeamais* was observed, the culture jars were not examined until 30 days after they had been set up, by which time both species had completed a generation and adults had begun to emerge. In order to determine the developmental stage of *S. cerealella* that killed the *S. zeamais*, another mixed culture was started and observed weekly for 4 weeks. Results obtained in this experiment showed that the significant weevil mortality occurred during the first week (Figure 2) suggesting that adult moths killed the weevils. Further investigations showed that neither physical contact between *S. zeamais* and *S. cerealella* nor a continuous presence of *S. cerealella* in the experimental jars for 2-4 weeks was necessary for weevil death. The latter implies that weevil adults that survived the 'moth effect' during the first week survived

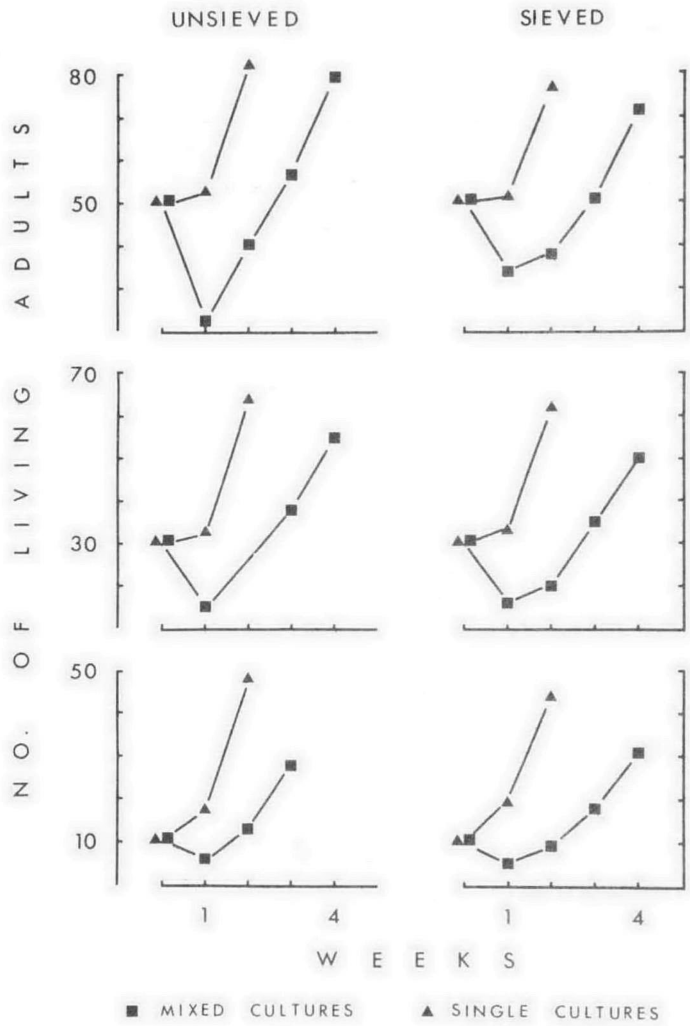

Figure 1. A comparison of the initial population growth of *S. zeamais* cultured alone (single) or with *S. cerealella* (mixed) using 10, 30, or 50 adult weevils. (The high initial mortality of *S. zeamais* is illustrated by the drop in growth recorded during the first week of census).

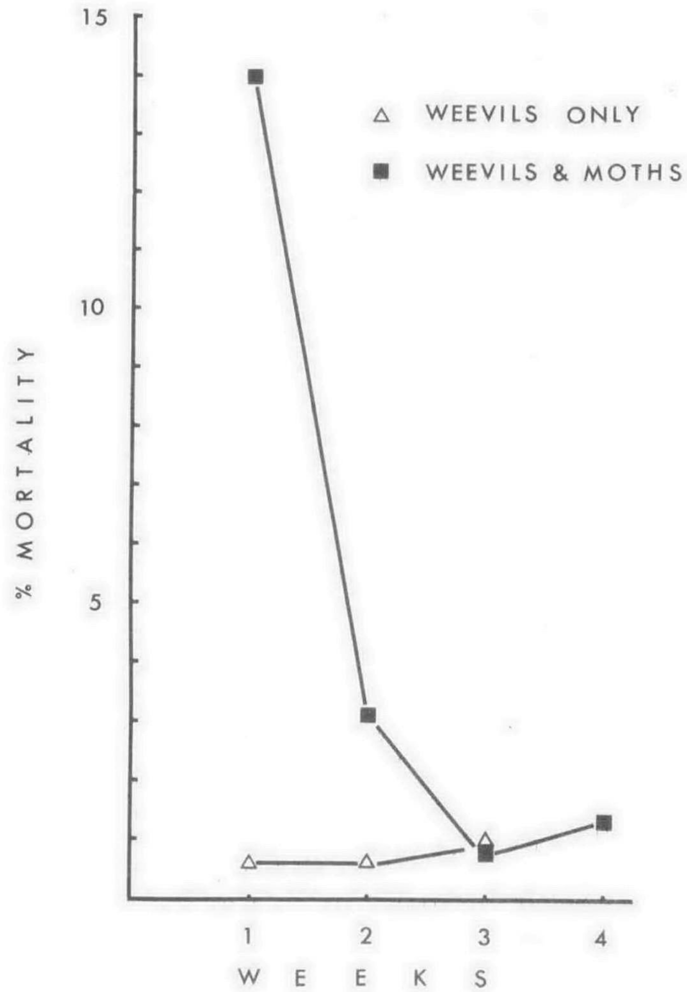

Figure 2. A comparison of the mortality of *S. zeamais* in single and mixed cultures with *S. cerealella* maintained over a four week period. (Scales exaggerated to emphasise differences in mortality)

subsequently, although they did not pass on this ability to their offspring. In all the above investigations, weevil mortality varied considerably between different replicates. It was also observed that older weevils succumbed more readily to the moth effect than younger weevils (Figure 3).

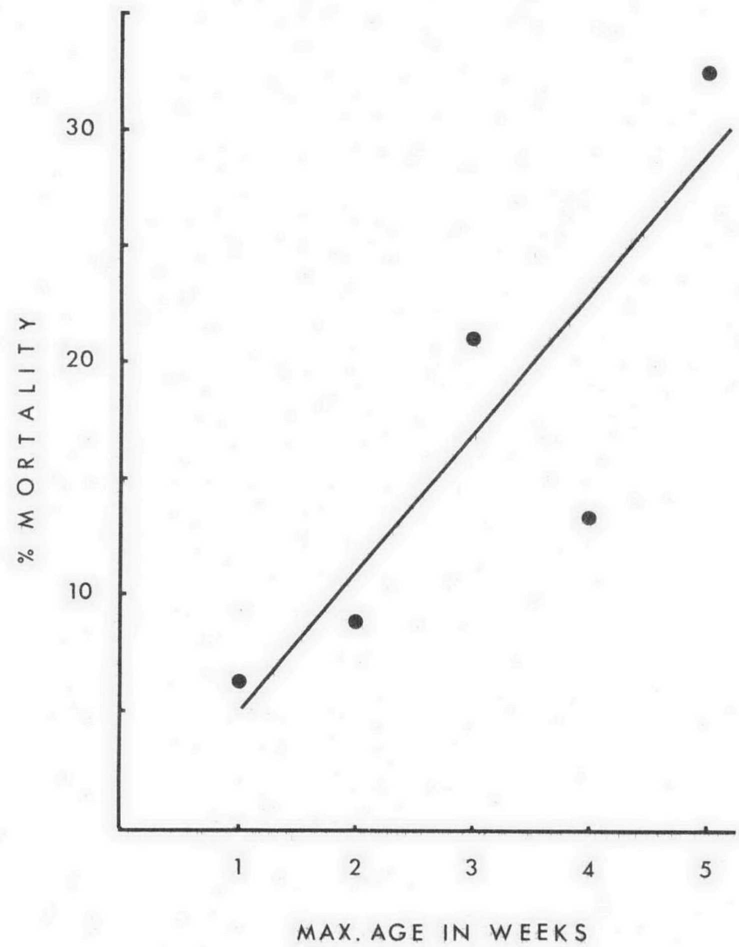

FIGURE 3. Effect of *S. cerealella* on different age groups of *S. zeamais*

Investigations into the effects of moth scales or a possible moth secretion on weevil adults: In the experiments in which *S. zeamais* and *S. cerealella* were placed together on maize grains, the weevils were in direct contact not only with the bodies of moths which died during the period but also with their body scales which they shed into the culture medium. If such dead moths and moth scales killed the weevils by hampering their movement, it would explain why mortality fell after one week of exposure as most of these were removed by sieving at the first count. On the other hand when *S. zeamais* and *S. cerealella* were physically separated by placing the former in tubes before being placed in a moth culture jar, weevil mortality was again high although scales and dead moths were excluded. In all cases the high weevil mortality was confined only to the first week of exposure. It was necessary therefore to determine more precisely whether *S. zeamais* was killed by moth scales or by a secretion released by *S. cerealella*.

About 25 g of maize was placed in each of 16 half litre glass jars grouped into 4 lots of 4 jars each. Eight batches of 200 freshly emerged *S. cerealella* adults were counted and each batch placed in a jar of maize for 3 days and removed by suction after shedding their scales in the food medium. At the same time about 1000 freshly emerged *S. cerealella* adults were confined in a litre jar for about 2 h during which they also shed their scales because of the intense crowding. Four batches of 200 moths each from this latter group were also placed in jars of maize for 3 days and then removed by suction. Moths in this group were referred to as 'rubbed' as they were placed on grain with most of their body scales already shed. The remaining 4 jars of maize were not seeded with moths and served as controls.

Twelve lots of 100 *S. zeamais* adults aged about one week were counted and placed on the above maize grains as follows: Four lots were placed in the 4 control jars (D), another 4 lots went to 4 of the 8 jars seeded with freshly emerged adult moths (A) and the final 4 lots to the jars seeded with 'rubbed' moths (C). One week later, a further 4 lots of weevils of similar age were counted and added to the remaining group of jars (B). The one week delay in group B was to find out if the number of weevils dying on moth-conditioned maize was affected by this delay.

All 16 jars were emptied 7 days after the introduction of *S. zeamais* and the number of dead and living weevils in each jar counted and the weevils discarded. At this count maize grains, moth scales and dead moths were returned to their appropriate jars and these were again seeded with a fresh supply of weevils. This procedure was repeated weekly for a further 2 weeks. In this way moth scales and dead moths were retained throughout the experiment in those jars in which these were present.

	Moth plus scale deposits (A)	Moth plus scales, delayed (B)	Moth plus few scales (C)	No moths or scales (D)
	36	29	21	5
WEEK	5	12	43	10
1	32	15	17	6
	7	4	29	7
Total	80	60	110	28
	5	17	4	2
WEEK	4	16	12	3
2	8	14	5	5
	3	18	3	3
Total	20	65	24	13
	7	9	4	6
WEEK	8	13	5	4
3	5	12	3	2
	8	13	6	5
Total	28	47	18	17

TABLE I. No. of weevils killed when 100 adults of *Sitophilus zeamais* were placed on maize grains conditioned by *Sitotroga cerealella* (see text).

The results of this experiment again showed that the high weevil mortality was still confined to the first week of exposure only (Table I). The cause of death of *S. zeamais* could therefore not be attributed to moth scales or dead moths. In fact during the first week of census, the largest number of dead weevils was from the group with fewer scales (C). The one week delay before introducing *S. zeamais* into the *S. cerealella*-conditioned maize did not reduce weevil mortality either. Indeed it raised it slightly but, unlike the other two groups, the mortality was spread fairly uniformly over the 3 weeks. It appeared therefore that the factor that killed *S. zeamais* in mixed culture with *S. cerealella* was probably a chemical which did not break down in one week in an undisturbed medium.

A scanning electron microscope study of *S. cerealella.* In most of the experiments described above it was found that adult weevils suffered their highest mortality during the first week of exposure to the moth and subsequent exposures did not appear to affect the weevil populations. It was also found that the lethal effects of moth-conditioned maize affected only those weevils exposed during the first week, few of those exposed in subsequent weeks dying. However if the moth-conditioned maize was left undisturbed for a week before *S. zeamais* was added, it remained lethal to the weevils. A microscopic examination of dead moths was therefore made using a Scanning Electron Microscope (SEM) to find out if any changes took place on the moth body after death, and which could be detected by this equipment, to explain the cause of weevil death.

Freshly emerged *S. cerealella* adults were anaesthetised with CO_2 and killed with ether and two pairs of each sex were immediately prepared for mounting. Another lot made up of two pairs of each sex were mounted one week later. Mounting was done onto aluminum stubs with silver dag and the specimens were coated with 400A gold using Polaran Sputter Coating Unit. Several shots were taken of different parts of the body of both sexes of the two lots of moths. Examination of the micrographs showed that different scale types were present on different parts of the body but no differences could be detected between those mounted soon after death and those mounted one week later in either sex. However, at the higher magnifications (x14,000 and above) it was not possible to obtain sharp focussing at certain areas of the body (Figure 4). These areas of obscured vision appeared at several spots but were interrupted by clear areas which showed details of the microstructure of the scales. After the SEM operator made certain that the blurred areas were not due to a technical fault, we concluded that these areas were covered by a secretion. I suggest that this secretion killed *S. zeamais*. The results of these experiments will therefore be discussed on this hypothesis.

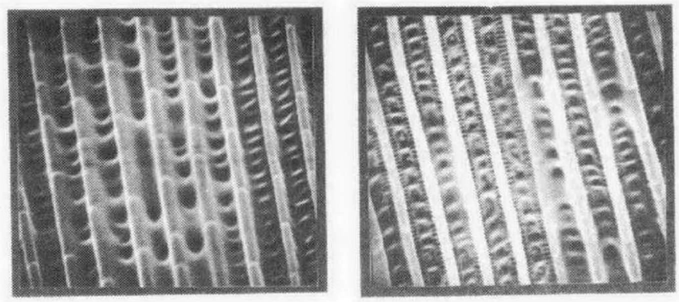

Figure 4. Scanning electron micrographs of wing scale of *S. cerealella* (Mag. x 14,5000) showing clear and blurred areas on scale.

DISCUSSION: In the 30 replicate jars in which *S. zeamais* and *S. cerealella* were cultured together, the mortality of weevils recorded 30 days after the beginning of the culture ranged from 20 - 100%. In 14 of these jars, weevil mortality was 50% and above. In 40 comparable jars in which *S. zeamais* was cultured alone weevil mortality over the same period ranged from 0 -20%. In 30 of these jars mortality was below 10%. The difference in weevil mortality in the two series of cultures was more than could occur by chance. Further experiments have confirmed this unusual weevil mortality. No similar observation has been reported before, although weevil populations have been reported to have been suppressed by *S. cerealella,* through crowding, resulting in a reduction in weevil oviposition (1). If *S. zeamais* was killed by a secretion from *S. cerealella* as I suggest in these experiments, then it should be possible to explain all the results obtained in these experiments on this premise. For the secretion principle to be tenable it should be able to explain the following main points arising out of the experimental results:

1. The variability in the results obtained,
2. The ability of the secretion to kill weevils not in physical contact with moths,
3. Why some weevils did not die after I week:
   a) among those that survived an initial exposure to the moths but remained on the moth-conditioned grain
   b) among a fresh supply of weevils placed on grain previously occupied by another batch of weevils,
4. The ability of the undisturbed moth-conditioned grain to kill weevils placed in it one week after setting it up.

From the results obtained in these experiments it appears that *S. cerealella* released a secretion which although it could be found on the body was probably present as a gas as well, or evaporated into gas with time. On release this secretion covered the scales of the moth and probably the food as well. An adult weevil which fed on a portion of grain on which this secretion was deposited would be killed. If a weevil fed on grain not covered by secretion, it survived. On the other hand in its gaseous form the secretion could kill weevils separated from moths by muslin and the likelihood of weevil death would increase if the weevils came to rest on the opposite side of the muslin close to where the moths released this secretion. Thus only weevils which fed on moth secretion or came close to the secretion would be killed. Under these premises the number of weevils dying in different replicates would vary as was found in these experiments. After about a week when nearly all the moths had died, weevils which survived the initial exposure to moths were more likely to survive because only a little secretion would remain, much of it having escaped from the medium through weevil activity. Thus when *S. cerealella*-conditioned grain was left undisturbed for one week, there was no loss of secretion and as many weevils placed on these were killed as those placed on freshly conditioned grain. When the medium has been stirred up by weevil activity for at least one week however, much of this secretion escaped and weevils which remained in the medium, or a fresh supply introduced into the stirred medium survived.

Although no further tests were carried out to determine the nature of the secretion reported above, two main gaseous secretions have been associated with stored product insects: quinones and pheromones. Quinones are secreted from specialised odoriferous glands. The principal constituent of this secretion was found to be ethyl quinone (2). This secretion is the cause of conditioning of the food medium in *Tribolium* (3) and probably the cause of conditioning by several other insects. Conditioning of the food medium has various effects on different insects (4, 5, 6). The other secretion common with insects is pheromones. These have been reported to be present in *S. cerealella* (7, 8). Unfortunately in the present investigations it was not possible to investigate the separate effects of males and females of *S. cerealella* on *S. zeamais*. Because of the variability reported in the results of these experiments, large numbers of moths were required at any one time for experiments. To obtain large enough numbers of each sex of *S. cerealella* for these experiments, large numbers of grains containing immature moths would have to be isolated before the moths emerged. It was not practicable to obtain such large numbers emerging within a few days of each other for experiments which could be satisfactorily replicated. The nature of the secretion or its source is therefore unknown but its effect on adult *S. zeamais* has been consistently found to cause their death.

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