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 NEW MUTANTS

A Tribolium castaneum

1. weird eggs. (wd): Autosomal recessive; linkage group unknown. Discovered in 1972 in a population derived from the Oregon synthetic strain. Egg surface is dry when laid and does not acquire a flour coating as do normal eggs. Phenotype of egg is determined by maternal genotype. See Dawson and Riddle (1975, J. Hered. 66: 31-32).
2. nude eggs (nd): Autosomal recessive; linkage group unknown. Discovered in 1973 in Oregon synthetic strain. Identical to weird eggs (wd) but not allelic.

 Report of P.S. Dawson

 NOTES - RESEARCH

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Bimodality of developmental period in Tribolium castaneum and Tribolium confusum

Earlier work in this laboratory demonstrated bimodal distribution of developmental periods in the Tribolium castaneum wild type (Sokal and Sonleitner, 1968; Sokal and Fujii, 1973). Experiments with T. castaneum bb and ss strains have also yielded varying degrees of bimodality (Kence, 1973). In the experiments reported here two further castaneum mutant strains, pearl and paddle were examined for this variable and a T. confusum mutant, McGill black was added to provide information on this related species.

Egg farms were set up for each of the four strais of Tribolium castaneum wild (++ Purdue University Foundation stock) as controls, pearl (University of Chicago), paddle (University of Chicago), and T. confusum Mcgill (University of California). After permitting the adults to lay eggs for three days, three replicates of the experimental cultures were set up in 8 g. flour at a density of 12 per g. to forestall crowding. The medium consisted of sifted whole wheat flour enriched with 5% brewer’s yeast. Cultures were maintained at 29.5 C and 70% relative humidity. After day 24, the pupae were sifted out at four-day intervals and stored in holding vials to prevent contamination. Adults emerging in the holding vials were removed and censused daily.

Bimodality in egg-to-adult development period is evident in the three castaneum strains. Agreement of the wild ++ results (Fig. 1a) with the previous work gives added support to the CS paddle (Fig. 1b) and CS pearl (Fig. 1c) findings. In contrast a unimodal pattern was demonstrated by the confusum beetles (Fig. 1d) which also took longer to develop.

The Kolmogorov-Smirnov two-sample test was used to test agreement of developmental period distributions within strains. No replicate differed significantly from any other one of the same strain. The same test was employed to compare distributions between pairs of strains. The results of the latter tests are as follows:

 All Pairwise Strain Comparisons

 n 1 n 2 D Critical value (P .05)

paddle vs. pearl 265 214 .1700 .12499

wild vs. paddle 266 265 .1867 .11804

wild vs. pearl 266 214 .1932 .12489

McGill vs. wild 201 266 .6201 .12710

McGill vs. pearl 201 214 .7966 .13359

McGill vs. paddle 201 265 .7901 .12721

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Reproductive Filure of Females of Genetic Strain bI of Tribolium confusum in
Grosses with Other Strains

Of the four “productivity strains” of Tribolium confusum developed by Park et al. (1961), bI is the most productive line. Later assays revealed that bI is also the least voracious of the T.confusum stoks that have been examined for cannibalism (Park et al. 1965).

In 1971, more than 15 years after the bI strain was isolated, we made reciprocal crosses between this line and the bIV and T. confusum “Chicago” strains. In five pair-matings, each involving a bI female and a male from another line, no progeny were recovered. The reciprocal crosses were all successful. We then attempted mass matings of 100 bI females to 100 bIV males, and, in another trial, to 100 “Chicago” males. In a year’s time, with frequent inspection, no progeny were recorded. Further crosses using bI females with b II or bIII males were similarly unproductive. We have since created F1, F2, and F3 hybrids using bI males and “Chicago” females, ad again te bI females proved reproductively incompetent with any of the hybrid males. Moreover, in more than 50 pair matings each, the hybrids could usually mate successfully in self-ings or in the other possible test crosses. Thus, if there are “bI-like” recombinants, they must be rare.

Females of bI appear to engage in normal copulatory behavior with males of all strains tested.

Stanley (1961) reported a similar infertility on the part of T. confusum “McGill Black” females with males of two other strains and the three possible hybrids (also see Slatis 1964). Since the bI results are so phenomenologically similar to Stanley’s report, this suggests a recurrent mechanism of sexual isolation.

We are attempting to analyze the genetic basis for bI infertility, and Dr. Terrence J. Enis of the Insect Pathology Research Institute, Canadian Forestry Service, Sault Ste. Marie, Ontario, is examining the material cytogenetically. If available for comparative studies, we would appreciate receiving a culture of T. confusum “McGill Black” beetles that exhibit the same sterility characteristics reported by Stanley (1961).

The results reported here were obtained at a climate of 29 0 C, 70 R.H.% in the standard flour medium described by Park (1948).

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Development of Tribolium castaneum Herbst larvae on different foods, their survival under starvation and longevity of the adults at various temperatures and relative humidities

Studies on different aspects of the biology of Tribolium castaneum Herbst were carried out in the Department of Entomology, Punjab Agricultural University, Ludhiana (Punjab), India. Fifteen newly emerged larvae (0-12 hrs) were kept in eah of the specimen tubes containing 15 g of the conditioned food. The development of larvae on each food was assessed on the basis of growth index, obtained by dividing survival percentage of larvae by average duration of larval stage in that food. The food materials in order of their preference in the descending order are crushed wheat grains = whole wheat flour = wheat bran Suji maida whole wheat grains. Speed of development was faster at 13 and 15 per cent moisture content of the food material.

Survival of larvae and adults under starved conditions:

Twenty larvae (6-7 mm long) and 20 young (0-12 hours) adults were kept without food in Petri dishes. The larvae were found to survive for 9.8, 6.1, 5.4, 5.5 and 4.3 days at 20, 25, 30, 35 and 40 0 C, respectively, the corresponding figures for adults were 25.8, 10.8, 10.0, 7.6 and 5.7 days at the respective temperatures and relative humidities.

Longevity of adults at different levels of temperature and relative humidity:

The duration of pre-oviposition period varied inversely with the temperature and relative humidity whereas the oviposition period varied inversely with the temperature and directly with the relative humidity. The duration of pre-oviposition period was 5.3, 4.0 and 3.7 days at 25, 30, 35 0 C and 4.7, 4.2 and 4.0 days at 40, 70 and 85 per cent relative humidities, respectively.

On an average the duration of oviposition period was 189.2, 131.5 and 55.9 days at 25, 30 and 35 0 C, and 62.4, 156.8 and 157.4 days at 40, 70 and 85 percent relative humidities, respectively. At 40 0 C there was no oviposition. The longevity of male and female was 239.4, 150.0 and 61.4 days and 194.8, 137.3 and 59.5 days at 25, 30 and 35 0 C, respectively. But at 40, 70 and 85 per cent relative humidity, the length of life was 71.0, 191.2 and 188.6 days and 67.2, 162.0 and 162.4 days in case of male and female, respectively. At 40 0 C, insects survived only for a few days at various levels of relative humidity.

It was found that in old females, the rate of egg laying and total fecundity was lower than that of young females when both of them were paired with young males. Further the age of the male did not affect the reproductive capacity of the young females. The rate of egg laying of old female (x young male) and young female (x young male) was 3.6 and 4.8 eggs per day per female and the total fecundity being 594 eggs and 731 eggs, respectively.

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Comparative study of egg cannibalism sites in Tribolium castaneum

Englert and Thomas (1970) reported differences in egg cannibalism rates by adults for two strains of T. castaneum, wild type vs. antennapedia. One of the possible reasons cited for the reduced rate of cannibalism by the antennapedia strain was a difference in tunneling behavior in the culture medium.

Fractionable shell vials patterned after those of Ghent (1966) were used. Twelve adult beetles (6 and 6 ) 26 days of age were introduced into each vial filled with 8 gm standard culture medium which had been “seeded” with 160 marked eggs. Each adult genotype was exposed singly to eggs of each genotype. After 24 hours, the vials were dismantled and marked and unmarked eggs were counted in each section. Overall cannibalism rates were recorded and compared, as well as section rates.

The overall egg cannibalism rates were calculated using the modified formula of Sonleitner (1961) and are presented in Table 1. The rates recorded for antennapedia are considerably lower than those recorded for wild type. This reduced egg cannibalism for antenapedia is consistent with that reported by Englert and Thomas (1970).

The question of whether the egg cannibalism rates, though different, could be attributed to possible tunneling behavior, which in turn would influence the number of egg encounters, was examined by comparing the number of eggs cannibalized in each section. Assuming that egg encounters would be random, thus, cannibalism would be uniform throughout the vials, and since the eggs were homogeneously distributed throughout the medium in eah vial section, a X 2-analysis was employed. The results are presented in Table 2.

In both egg treatment groups, the antennapedia beetles are seen to be cannibalizing in a “nonuniform” manner, while the wild type beetles are randomly cannibalizing in
a uniform manner. The antennapedia beetles appear to “prefer” the surface areas for movement. However, even with this discrepancy of movement, there appears to be a real difference in egg cannibalism rate that is not totally explained by movement behavior.

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Table 1. Egg cannibalism rates a for antennapedia and wild type adult
 T. castaneum. (Mean + standard error).

Egg Adult Genotype
Genotype +/+ ap/ap

ap/ap 3.41 + 0.70 2.49 + 0.29

+/+ 4.34 + 0.44 1.28 + 0.32

a e x 10 2

Table 2. Chi-square analysis for number of eggs cannibalized by antennapedia and
 wild type adult T. castaneum per vial section.

Treatment a Vial Section X 2 and P values
 S I II III IV

ap on ap O 24 57 40 30 54 X 2 = 29.16

 E 10.25 48.69 48.69 48.69 48.69 P .001

ap on + O 18 36 33 24 13 X 2 = 34.55

 E 6.20 29.45 29.45 29.45 29.45 P .001

+ on + O 12 78 77 84 80 X 2 = 1.67

 E 16.55 78.61 78,61 78.61 78.61 P = .80

+ on ap O 14 75 52 72 76 X 2 = 5.59

 E 14.55 68.44 68.44 68.44 68.44 P = .24

a
 adult genotype listed first.

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Interspecies competition with one competitor rare.

Populations made up of different proportions of Tribolium individuals from the McGill confusum stock and the wild-type castaneum stock were set up to determine whether there are any density-dependent effects upon the outcome and progress of competition between the two species. Discrete generations were imposed upon the system to intensify the results of the competition process. Although circumstances beyond my control caused the experiments to be terminated after at most four generations, results emerged which might be followed up by other workers.

Discrete generations were imposed by having the desired number of adults of the two species lay eggs for a three-day period in 8 g of flour. The eggs were then sifted from the flour and spread randomly over 8g of fresh flour in a new vial. The adult population that resulted was censused after five weeks, or later if there were more than five or six larvae remaining in the flour. These adults then formed the egg farm for the next generation. This procedure is basically similar to that of Sokal, Kence and McCauley, 1974.

One control replicate was started with 400 adult individuals for each species, and five replicates were set up with 200 individuals of each species in the initial egg farm. In addition, five replicates each were set up with one or the other species rare. In the rare species, three males and three females of the species were sexed as pupae, allowed to pupate, and then given an additional period of one or two days to mate in 1 g of flour. These adults were then added to 400 adults of the common species for the three-day egg-laying period. This method insured that the rare species would have some female members capable of laying eggs. The cultures were maintained at 29.5 0 C and 70% R.H.

Figure 1 shows the results of these experiments for several generations. In each interspecies competition experiment, the confusum population decreased and in some replicates had ecome extinct when the experiment was terminated. In contrast, the castaneum population increased when rare, common, or at equal proportions. In the controls the castaneum population oscillated around 450 adults, while confusum declined sharply to about 150 individuals.

The decrease in confusum population size in each experiment reflects its low egg production over a three-day period. About 400 eggs were counted from 220 individuals after three days in 8 g of flour. In contrast, 1150 eggs were counted from the same number of castaneum. Sokoloff and Ho (1962), uing similar techniques for a single generation with five pairs of beetles, recorded egg production for ech replicate. In 5 g of flour, the castaneum replicates produced 202 eggs and the confusum replicates produced 125 eggs on the average. The greater density of a population of 220 individuals, then, lowers the egg production per individual for both species and increases the difference between the two species. Since the cannibalism of eggs at the low density of ten individuals per 5 grams is probably negligible, there is clearly a difference in oviposition rate of the two. In addition, when confusum and castaneum were initiated in equal numbers of 200, confusum decreased and castaneum increased even though the rate of cannibalism on both egg types would presumably be the same.

The lower egg production of confusum is due in part to its lower ovipositon rate but cannibalism may also be important in creating differences between the two species at high densities under this husbandry. The effect of egg cannibalism on population size is magnified by the discrete generation husbandry of this experiment. Under continuous generations the pre-adult stages would have a greater dispersion over time ad a larger adult population could be maintained with the same voraciousness of cannibalism of adults on eggs in the population. Under discrete generations, the carrying capacity is lower for a species with a high degree of cannibalism. This is a possible contribution tot the low equilibrium value for confusum shown in the control and the greater effect of density on confusum egg production compared with that of castaneum.

Adult weight of individuals of either species generally changed inversely with respect to population size change. The magnitude of the change was usually 10% or less. Schlager (1962) has shown, using castaneum, that adult weight is inversely related to popula-density. This relationship suggests that when the adult population is below capacity many eggs are produced and survive because of less cannibalism but that this excess number of individuals faces limiting resources as larvae with a resulting lowering of adult weight per individual. When the adult population is high, few eggs survive due to increased cannibalism and decreased egg laying, but those that make it are less restricted by resources as larvae, so that adult weights go up. When the population is slightly different from its capacity, then, the difference in egg number creates a change in adult weight rather than a change in mortality, permitting fluctuation in numbers of adults around the carrying capacity.

There is no evidence from these experiments that the rare species is afforded any advantage by being rare. It would be interestingto re-do the experiment with castaneum as the rare species without putting confusum at the disadvantage of being well above its capacity initially. Thirty-two g of flour with 400 confusum and six castaneum would be
an effective set-up.

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Habitat selection by the unsaturated fatty acid sensitive mutant of tribolium castaneum

Introduction

An important component of the life history of any animal is where it lives. Since habitats differ with respect to their desirability to particular animals, it should be possible to observe an animal choose between habitats differing in desirability. In the presence of such environmental variations, natural selection is expected to lead to an ability to recognize and select favorable habitats (King & Dawson, 1973). The effects of habitat selection may be a significant determinant of the degree of specialization or niche breadth of a population or species.

In this paper, we have investigated the habitat selection of the corn oil sensitive (cos/cos) mutant of Tribolium castaneum (Costantino, Bell & Rogler, 1967) in environments varying in space and time. Other studies have been concerned with the effects of temperature and humidity gradients on T. castaneum (Amos & Waterhouse, 1969), conditioned and fresh media (Chent, 1963), and food and temperature gradients on T. castaneum and T.confusum (King & Dawson, 1973). The present study differs from these studies primarily by considering the distribution of beetles in heterogeneous environments over time.

Materials and Methods

Populations were started by placing 12 adult T. castaneum cos/cos of each sex into 12x17 cm rectangular plexiglass boxes containing 100 gm of medium. The series of boxes were divided into four types of environment differing in the amount and location of “standard” (95% whole wheat flour, 5% dry brewer’s yeast) and “corn oil” (90% whole wheat flour, 5% dry brewer’s yeast, 5% liquid corn oil) medium. The four types of environment were: (1) all corn oil medium (CO), (2) all standard medium (STD), (3) half the container consisting of standard medium, the other half containing corn oil medium (1/2), (4) alternating quarters of standard and corn oil medium (1/4). A total of 48 boxes were prepared; 12 of each environment. All containers were maintained at 92 0 F and low (40-60%) relative humidity. Sampling was performed at 3 week intervals for a total of 12 weeks. During each census period, 3 containers of each environment were sampled by counting the number of larvae, pupae and adults in each quarter of the container. After censusing a container, it was discarded. The resulting data consisted of information on the distribution of life stages in each environment at 3, 6, 9 and 12 week intervals.

Results

The sum of the numbers of each life stage found in each quadrat for 3 observations is given for the four environments over time (Table 1). The homogeneous environments (CO, STD) were characterized by fairly even numbers in each quadrat for all life stages except for pupae in corn oil on 2 occasions. Adults appeared to be evenly distributed in heterogeneous environments (1/2, ¼) as well. However, both pupae and larvae were quite aggregated in these environments, usually living in the standard medium section of the container.

The total number of animals in each life stage was clearly related to environment
(Table 2). Beetle density was lowest in CO, greatest in STD, and achieved intermediate levels in the two heterogeneous environments.

A common method of analyzing the spatical dispersal of a population is to compute the coefficient of dispersion (Elliott, 1971, Greig-Smith, 1957, Pielou, 1969, Stitler & Patil, 1971)..

This index is based upon the variance-to-mean ratio, which has been used as an indicator for 3 general classes of dispersion: random uniform and aggregated.

 s 2 (N-1) s = sample variance
C.D. =
 x x = sample mean

 N = number of quadrats sampled

The index approximates a chi-square distribution with N-1 degrees of freedom (Elliott, 1971), which allows significance testing for deviations from a random distribution. Use of the coefficient of dispersion has been criticized by Mead (1974) on the basis that it is sensitive to quadrat size, but in this study, the quadrat size remained constant, and the index was suitable for making comparisons between life stages and environments.

The coefficient of dispersion was calculated for each life stage in each environment for the four census intervals. (Table 3). Nearly every container showed significant aggregation, with much higher values for immature life stages in heterogeneous environments. There was a tendency for pupal and larval aggregation to diminish over time; adult dispersal varied in no meaningful pattern over the sampling periods.

A 4 x 4 x 3 factorial analysis of variance was performed on the coefficients of dispersion to test for differences between census dates, environments, and life stages. The analysis yielded significant (p. .05) differences between the main effects, all 2-way interactions, and the 3-way interaction. Naylor (1961) also used coefficients of dispersion as sample variates in an analysis of variance. The expected chi-square distribution of this index is a robustness of the F-test to permit its use.

Discussion

The T. castaneum cos/cos mutant is characterized by the inability of its larval stages to utilize unsaturated fatty acids in the diet (Costantino, Rogler & Bell, 1968). One might expect that these larvae would preferentially select standard medium over corn oil medium in environments where both media occur in discrete units. This selection was demonstrated (Table 1) where 72-91% of the larvae were found in the standard medium portion of heterogeneous environments (1/2, ¼). The distribution of pupae was more clearly difined: 87-100% occurred in the standard medium portion. Adults demonstrated no comparable habitat selection; 42-59% were found in the standard medium portion. It is obvious that the standard medium “habitats” were selectively chosen by larvae and pupae. Since the pupa is an immotile stage, it is likely that pupal distribution is the result of larval habitat selection for pupation sites. Since adults are distributed more randomly than immature stages, one might infer that eggs are laid randomly. Naylor (1959m k961) studied dispersal in adult T. confusum and
T. castaneum and reported a tendency for males to assume an aggregated distribution and females to be distributed more uniformly. If eggs are laid uniformly or randomly in
a heterogeneous environment, then many young larvae will find themselves in suboptimal corn oil habitats. At such small sizes, the dispersal power of the larvae is relatively small, so it may take several days before they locate the standard medium. Most large larvae should have found the preferable habitat, and become pupae there. The greater portion of pupae than larvae found in standard medium may be the result of size-dependent larval dispersal from corn oil to standard medium. Adult beetles tended to occur in an aggregated distribution, but the nature of the clumping does not appear to be directly related to food resources.

The tendency for the degree of aggregation of larvae and pupae to diminish over time is probably related to interactions among the animals. The first sample was taken when the populations were at low densities, and habitat selection could presumably occur without regard to crowding, cannibalism, etc. Subsequent samples were made at higher population densities, where more intense interactions may have influenced choice of optimal habitat.

The presence of unsaturated fatty acids in the environment noticeably affected the population growth of the T. castaneum cos/cos mutant (Table 2). Adult, pupal and larval stages achieved uniformly low densities in pure corn oil supplemented environments, and correspondingly greater densities in pure standard environments. Both heterogeneous environments were characterized by sustaining intermediate densities, with little difference between the geometry of the two environments.

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 Table 2

 Total number of each life stage in each environment over a 12-week period.

 ENVIRONMENT
 Life
 Week stage CO ½ ¼ STD

3 Adult 22 22 27 38
 Pupa 0 165 201 309
 Larva 70 816 684 1220

6 Adult 172 936 765 1439
 Pupa 25 45 75 60
 Larva 123 391 411 999

9 Adult 77 974 946 1482
 Pupa 3 41 35 10
 Larva 16 216 175 388

12 Adult 160 835 1074 1318
 Pupa 4 64 109 50
 Larva 53 244 302 360

\*Entry is the sum of 3 replicates over 4 quadrats.

 TABLE 3

Coeficient of dispersion calculated for adult, pupal ad larval life stages of t. castaneum cos/cos in four environmental patterns over a 3 month period.

 ENVIRONMENT

 Life
Week Stage CO ½ ¼ STD

3 Adult 13.11 23.22\* 9.11 12.37
 Pupa - 103.26\* 162.52\* 112.98\*
 Larva 29.30\* 366.30\* 188.96\* 343.23\*

6 Adult 69.11\* 38.24\* 36.88\* 89.97\*
 Pupa 18.11 72.63\* 82.82\* 22.15\*
 Larva 44.94\* 264.79\* 265.30\* 33.96\*

9 Adult 13.61\* 78.38\* 11.50 28.98\*
 Pupa 9.00 43.58\* 45.47\* 7.87
 Larva 12.31 79.83\* 72.85\* 7.48

12 Adult 27.88\* 51.63\* 80.62\* 66.60\*
 Pupa 6.67 66.01\* 125.19\* 10.48
 Larva 35.87\* 88.59\* 140.84\* 23.21\*

\*p .05 non-random aggregation

Entry is the sum of 3 replicates per environment.

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The heritability and genetic correlation of chaetae number on two pupal segments of tribolium castaneum (Herbst)

The genetics of chaetae number in Tribolium has largely been ignored by geneticists. This is probably due to the small size of the chaetae and the scarcity of mutants affecting them. On the other hand, the chaetae of Drosophila have been used extensively to investigate the genetic control of development (Rendel, 1967).

Lange and Bell (1970) reported on the genetic control of the development of the pregential chaetae located on the eighth pupal sternite. They reported that following eight generations of selection for the number of pregenital haetae there was
a change in the number of chaetae on other pupal segments. This change was not quantified and consequently we have been looking at the genetic variation that exists on other pupal segments. In particular, this paper concerns the fourth pupal abdominal tergite (hereafter called tergite).

The strain used for these studies was Purdue Foundation b maintained at Milwaukee as a random mating control stock. This strain is homozygous for black body color and as such the pupal chaetae are darker and easier to see than those in other backgrounds. A sample of 500 adults was used to lay eggs for 48 hours in standard 95% whole wheat – 5% nutritional yeast media. All stages were raised were chosen at random to be the parents of generation – 1. This was done because Purdue Foundation b is not maintained at 33 0 C and the number of pupal chaetae is influenced by temperature and maternal effects.

The chosen adults layed eggs in standard media for 48 hours. After days of culturing the first 500, male and female pupae were scored for the number of pregenital chaetae and the number of fourth tergite chaetae. These represent Generation O. A random sample of 50 male pupae were taken and each was mated to two females chosen at random. The females were then separated and layed eggs for 48 hours in vials with three grams of media.

Eighteen days later the first 10 male and female progeny (at most) from each vial were scored for both traits. These progeny represented generation 1.

The results of these two generations are presented in Table 1 below. First comparing the values for the pregenital chaetae to those reported by Lange and Bell (1970) for generations – 1 and 0, 6.2 + 0.04 for males and 5.6 + 0.04 for females, we can see that the males are nearly the same, but the females are lower in the present study. However the males have a greater number of pregenital chaetae than the females as before.

Table 1

Number of progeny and mean chaetae number for two unselected generations

 Males Females

 Pregenital Tergite Pregenital Tergite

Gene- No X + S.E. X + S.E. No. X + S.E. X + S.E..
rations

 0 467 6.239 + 0.04 46.43 + 0.50 500 5.13C + 0.04 50.07 + 0.5
 1 683 6.175 + 0.04 45.91 + 0.40 643 5.185 + 0.04 42.88 + 0.4

The chaetae on the tergite are much more numberous than the pregential chaetae (Table 1). The females have a significantly ( = 0.05) greater number of chaetae on the tergite than do the males (t = 4.88 and t = 3.46 for the generation 0). It is not known whether this is a general phenomena for the pupal tergites or a phenomena for this tergite only.

The phenotypic correlation between these two traits is given in Table 2. It is a very low correlation which is not statistically significant in generation 0 ( = 0.05) but is statistically significant in generation 1. If both generations are combined, the resulting correlation is also significant. Thus there would appear to be a small positive phenotypic correlation between the number of tergite and pregenital chaetae.

The ANOVA’s used to estimate the heritability and genetic correlation for the pregenital and tergite traits are presented in Table’s 3 and 4. Using these, estimates of the heritability and genetic correlation for the two traits were calculated and are given in Table’s 5 and 6.

Looking first at the pregenital chaetae, it can be seen that the heritability is lower in males than it is in females and that the dominance variance or the common environmental variance is greater than the additive variance. Since the pregenital chaetae exhibit only a small amount of dominance, if any, and have a large maternal effect which inflates the dam estimate of heritability.

For the chaetae on the fourth abdominal tergite, it can be seen that the sexes have about the same heritability. Further the additive variance is greater than any dominance or maternal effects

The genetic correlation is greater in the males than females and there appears to be more dominance or maternal effects in female progeny than in males.

In general, these estimates of heritability support the contention of Lange and Bell (1970) that the development of chaetae is greatly influenced by the sex of the individual. Further, the action of the genes controlling chaetae development are drastically modified as to dominance relations, maternal effects, and genetic correlation dependent upon the sex of the individual.

Table 2
Phenotypic correlation between pregenital and tergite chaetae number.

 Males Females

Generation No. r + S.E No. r + S.E.

 0 467 0.041 + 0.046 500 0.044 + 0.045
 1 683 0.221 + 0.037 643 0.127 + 0.039
 Total 1150 0.15 + 0.029 1143 0.09 + 0.029

Table 3
ANOVA for heritability and genetic correlation estimates for males.

 Mean Squares

Source df Pregenital Tergite Correlation

Sires 38 1.447 386.394 25,331.008
Dams/Sires 39 1.206 119.079 8,496.912
Progeny/Dams/Sires 525 0.938 72.632 4,226.725

Table 4
ANOVA for heritability and genetic correlation estimates for females.

 Mean Squares

Source df Pregenital Tergite Correlation

Sires 41 2.270 419.595 19,015.003
Dams/Sires 42 1.422 155.478 9,644.905
Progeny/Dams/Sires 502\* 0.916 81.328 4,388.005

\*two females were not classified for tergite number and thus df for Progeny for tergite and the genetic correlation is only 500.

Table 5
Heritability and genetic correlation estimates for males

 Pregenital Tergite Correlation

Sire Estiate 0.049 0.696 0.705
Dam Estimate 0.154 0.277 0.414

Table 6
Heritability and genetic correlation estimates for females

 Pregenital Tergite Correlation

Sire Estimate 0.187 0.618 0.373
Dam Estimate 0.320 0.452 0.611

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Food preferences of the merchant grain beetle,
Oryaephilus Mercator Fauvel

Eighteen stored products including processed cereals, oil-seeds, nuts, and raisins were placed in circular chambers designed to test insect feeding preferences. The chambers were described and used with stored products insects by Loschiavo (1952, 1959). The relative position of each food in the sectors of each chamber was selected at random. Different foods were used in each of three tests.

In Test 1 the foods were: rolled oats, shelled sunflower seeds (confection variety Commander), shelled sunflower seeds (oil-seed variety Armavirec), flaxseed (Noralta), walnuts (broken kernels), wheat germ, rapeseed (whole seeds, Zephyr), Sultana raisins, breakfast cereal consisting of flax, rye and wheat, shredded, un-sweetened coconut, un-enriched flour, and flour plus 5% brewers’ yeast. In Test 2 six foods from Test 1 were replaced by brown rice, crushed soda cracker, crushed corn flakes, bread crumbs, bran, and pancake mix. In this test the rapeseed was milled in a blender to a meal-like consistency. In Test 3 the foods were: sunflower seeds (oilseed variety Armavirec), rolled oats, brown rice, flaxseed (Noralta), walnuts (broken kernels), and crushed soda crackers.

Filled chambers were placed in a room at 30 0 C and 65% RH, and held at these conditions for two days before the introduction of 200 adults of the merchant grain beetle, Oryzaephilus Mercator, Fauvel onto a central stage. The beetles were allowed a “setting” period of one hour on the stage before being allowed access to the foods in the various sectors. After 48 hours, the contents of each sector were removed manually and by aspiration. The beetles were separated by sieves and counted.

Results and Discussion

In the three tests shelled sunflowers and rolled oats wee the most preferred foods (Tables 1, 2, 3). The preference for sunflowers is not readily obvious in Table 1 where 35% of the beetles were distributed about equally between two varieties. The percentages found in sunflowers in Tests 2 and 3 were 35% and 33% respectively (Tables 2 and 3). In all three tests about 24% of the beetles were found in rolled oats. Thus, 57 to 60% preferred these two foods.

O. Mercator is associated with oil seeds (Howe, 1956, Loschiavo and Smith, 1970; Verner, 1971), and it is noteworthy that the two most preferred foods in these tests are high in oil content. Sinha (1972) found that several varieties of sunflowers, including Armavirec, supported the development and fecundity of O. Mercator. In light of his results and those observed in this study there seems to be a relationship between feeding preference and the ability of a food to support development.

Few or no insects were recovered from whole or ground rape-seed (Tables 1 and 2) which also has a high oil content. The variety used, Zephyr, is low in erucic acid, but it is not known whether this or some other component(s) in rapeseed repel merchant grain beetles or deter feeding. The suggestion that a volatile repellent may be present in rapeseed is supported by the finding that only a few beetles were found in whole seed (Table 1) and none in ground rapeseed (Table 2). The greater concentration likely to be present in the ground seed could account for the absence of insects.

Beetles were found in nearly equal numbers in walnuts in the first two tests, and slightly higher numbers in Test 3. In all tests walnuts ranked fifth. Flaxseed and wheat germ contained 22.0 and 14.0 beetles respectively in Test 1 and 11.0 and 4.0 respectively in Test 2. The higher recovery of beetles from foods of low oil content, for example, brown rice, than from walnuts or wheat germ, (Table 2) indicates that oil content is not the only factor affecting feeding preference by this species.

No beetles were found in un-enriched flour, flour plus 5% brewers’ yeast, or shredded coconut (Table 1). Their absence in a high oil content product like coconut is further evidence that choice of food by merchant grain beetles is not governed by oil content alone. In Test 2 bread crumbs, bran, and pancake mix were the least preferred of 12 foods (Table 2).

Shelled sunflower seeds, rolled oats, and brown rice were the highest ranking foods in the second and third tests (Table 2, 3). Crushed soda crackers ranked fourth out of 12 in Test 2 (Table 2) and sixth out of six in Test 3 (Table 3) but in both tests the numbers in this food were almost identical. Flaxseed ranked sixth and fourth in Tests 2 and 3, respectively, (Tables 2 and 3), and fourth in Test 1 (Table 1).

In these tests the number of insects found in a particular food was not influenced by the food in adjacent sectors. Nevertheless, considerable variation occurred between replicates and its source must be investigated.

General Discussion

The results show that adults of O.mercator have distinct food preferences for shelled sunflower seeds and rolled oats, and confirm results of other workers who observed that this insect is associated with foods of high oil content. However, oil content per se does not determine choice because at least one preferred food namely, brown rice is not high in oil content, while some that were not preferred, namely, rapeseed and coconut have high oil contents. It is postulated that rapeseed may contain volatile compounds that repel beetles. Sinha (1972) found that the rapeseed variety Target was satisfactory for the development and fecundity of merchant grain beetles, but another variety (Brassica napus type) whether whole or crushed, failed to support this species. Verner (1971) found that the order of preference of four kinds of oilseed by O.mercator was sunflower seed, groundnut (peanut), rapeseed, and soya bean.

The establishment of O.mercator as a pest of rapeseed in storage probably will depend, to some extent, on chemical and physical factors in a particular variety.

Acknowledgement

I am pleased to acknowledge the technical assistance of Mr. B. Salamon.

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Table 1. Distribution of 200 adults of O.merator in food-preference chambers
 containing 12 foods.

 Number of
 beetles\*
 Food 1 2 Mean

Rolled oats 42 52 47.0
Shelled sunflower (confection) 27 45 36.0
Shelled sunflower (oilseed) 37 32 34.5
 Armavirec
Flaxseed (Noralta) 27 17 22.0
Walnuts (broken) 11 18 14.5
Wheat germ 16 12 14.0
Rapeseed (whole) 12 4 8.0
Raisins (Sultana) 6 8 7.0
Cereal (flax-rye-wheat) 2 2 2.0
Coconut (shredded unsweetened) 0 0 0
Flour (un-enriched) 0 0 0
Flour (+5% brewers yeast) 0 0 0

\*Pooled = 369.78; , 0,001 = 24.72.

Table 2. Distribution of 200 adults of 0.mercator in food-preference
 chambers containing 12 foods.

 Number of beetles\*
 1 2 3 4 Mean

Shelled sunflower (oil seed,
 Armavirec) 73 67 67 80 71.8
Rolled oats 27 58 62 40 46.8
Brown rice 23 20 20 25 22.0
Soda crackers (crushed) 8 21 23 6 14.5
Walnuts (broken) 27 3 6 15 12.8
Flaxseed (Noralta) 13 6 5 20 11.0
Corn flakes (crushed) 5 14 6 5 7.5
Wheat germ 6 5 2 3 4.0
Bread crumbs 4 0 0 1 1.3
Bran 2 0 2 0 1.0
Pancake mix 0 1 1 0 0.5
Rapeseed meal 0 0 0 0 0

\*Pooled = 1313.76 0.01 = 24.72.

Table 3. Distribution of 200 adults of 0.mercator in food-preference chambers
 containing 6 foods.

 Number of beetles\*
 Food 1 2 3 4 Mean

Shelled sunflower (oilseed) 53 58 74 77 65.5
Rolled oats 66 32 48 46 48.0
Brown rice 28 46 22 18 28.5
Flaxseed (Noralta) 11 39 17 16 20.7
Walnuts (broken) 29 10 18 20 19.2
Soda crackers (crushed) 9 15 14 18 14.0

Pooled = 245.01; , 0.01 = 15.08

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A carbon-14 labeled glucose method of measuring respiration rate and approximate food intake in Tribolium

Introduction

This method was developed as part of a series of experiments to study metabolic differences among body weight selected lines of Tribolium castaneum (Medrano and Gall, 1973).

The method consists of feeding radioactive U-C 14 –glucose labeled flour to Tribolium larva and measuring the radioactivity in respired CO 2 and in the body tissue after a period of incubation. Respiration rate and the relative rate of glucose utilization is estimated from the CO 2 values. Feed intake is approximated from the total measured radioactive carbon.

Preparation of Diet

The test diet was prepared using our standard laboratory Tribolium medium, i.e. 90% unbleached white wheat flour and 10% dried brewers yeast. A concentration of radioactive label in the diet of 0.50 uc (micro-curies) per 100 mg of diet was found to be appropriate to use in our experiments.

Ten grams of diet are prepared by mixing 0.05 ml of a 20% ethanol solution, containing 1.0 mc (milli-curie) of U-C 14 –glucose per ml, with 1.0 ml of 95% ethanol and then preparing a slurry by mixing the liquid with 1.0 g of flour. The radioactive slurry is dried in an oven at 56 0 C for approximately 90 minutes, then gently powdered with a spatula and passed through a 60 mesh sieve. The remainder of the diet, 8.0 g of flour and 1.0 g of yeast, is added and thoroughly mixed by tumbling the diet in a glass jar. Obviously, the yeast or the complete diet could be labeled, depending on the desires of the experimenter, by modifying the material used in preparing the slurry.

Feeding Trials

It is desirable to obtain the larval material from short, 8-10 hour, egg-collection periods in order to minimize variability in weight and developmental stage.

Larvae of the desired ages, generally 8, 10, 12, 14 and 16 days, are weighed in groups of 50 to 15 individuals, depending on size, to obtain samples of approximately 25 mg. The larvae are then placed in 25 ml Erlenmeyer flasks, containing 100 to 150 mg of labeled diet which have been equilibrated in an incubator at 33 0 C and 70% relative humidity for at least 12 hours. Each flask is then sealed with a self-sealing rubber serum cap fitted with a plastic center-well (Kontes Glass Co.) and returned to the incubator. The incubation is continued for additional periods of from 1 to 6 hours. It is advantageous to place a 1.5 cm square piece of Whatman 1 filter paper in the center-wells to increase the surface area of CO 2 absorption. Generally, four replicate samples were used for ech age and hourly incubation period.

Fifteen minutes before the end of the incubation period, 0.2 ml of hydroxide of hyamine-10x (Packard inst.) are injected into the plastic center-well to collect the CO 2 produced. (It is essential that the OH-hyamine is placed only in the center-well and the tip of the injecting needle should be wiped against the filter paper in the center-well.) The incubation is terminated by injecting 0,2 ml of chloroform in 1.0 ml of 1 N sulfuric acid into the flask. This mixture kills the larvae instantaneously and liberates any CO 2 held in the media. The flasks are then allowed to sit at room temperature for one hour, with an occasional slow shaking, to permit the hydroxide of hyamine to effectively trap the liberated CO 2.

Measurement of CO 2 and Food Intake

The center-wells are carefully removed from the rubber caps and placed in a 25 by 55 mm scintillation vial. Then 10.0 ml of scintillation fluid (4.0 g of omniflour (New England Nuclear) per liter of toluene) are added, the vials vigorously shaken and then allowed to sit for 48 hours prior to counting to dissipate residual photo-luminescence.

The larvae in the incubation flask are washed, with distilled water, onto a 9 cm square piece of cheese cloth placed over a 25 mm funnel. The larvae are thoroughly washed with water, placed on a paper towel and allowed to air dry. The dried larvae are placed in a scintillation vial containing 2.0 ml of a 3:1 mixture of hydroxide of hyamine-10x and 30% KOH. After a 24 hour period of initial digestion at room temperature, the samples are heated with occasional shaking for 45 minutes at 70 0 C, allowed to cool, five dros of 30% hydrogen peroxide added and again reheated for 30 minutes. After the samples cool to room temperature, 1.0 ml of glacial acetic acid and 10 ml of scintillation fluid for aquous samples (5.0 g of omni-flour in 500 ml of Triton-X-100 (Rohm and Haas) and 1000 ml of toluene) are added. The samples are then allowed to sit for 48 hours and then counted with a liquid scintillation spectrometer.

Respiration rate can be estimated from the CO 2 produced as:

R.R. = cpm\* CO 2 / hour / mg-weight \*(counts per minute)

Assuming that all the radioactively labeled carbon ingested was absorbed by the larvae and excreted only as CO 2 during the short incubation, the total intake of C 1 4 (cpm) will equal the sum of cpm from CO 2 and cpm from larvae. Then the total food intake in ug per larvae, can be approximated as,

 total intake of C 14 (cpm/larvae)
 Approx. food intake =
 specific activity of diet (cpm/ug)

Results and Discussion

Table 1 shows some experimental data collected on a Tribolium castaneum control population (BC1-2-1C), that had been randomly selected for 21-day pupa weight for 54 generations (Gall, 1971).

Respiration rate expressed as cpm of CO 2 per mg net weight tends to decrease with age. In most of our trials the decrease was smooth and continuous down to 16 to 18 days of age. At these later stages the larvae consume very little food and begin their metamorphic changes towards the pupal stage. In the example shown in Table 1, respiration rate at 14 days of age was a little higher than expected, which serves to point out that the method is subject to some degree of variation due to factors that influence the ad-libitum intake of food by the larvae.

As pointed out earlier, to minimize the variability of the results it is important to: a) use larvae from short egg-collection periods, to obtain organisms that are similar in stage of development and b) to prevent any prolonged temperature and humidity shock to the larvae by rapidly returning the incubation flasks to the incubator once they are sealed.

 TABLE 1

 Respiration rate and approximate food intake at 10, 12, 14 and 16 days
 of age in Tribolium castaneum control line BC1-2-1C

Age Larva CO 2 Body Total C 14 Food
 Weight Respiration intake intake

Days mg cpm/mg cpm\* cpm cpm mg/24 hr

10+ .178 + .005# 213+14 37+2 18+1 56+3 0.159

12 .360 + .010 118+11 42+4 33+3 76+6 0.216

14 .873 + .019 138+7 120+6 160+7 280+11 0.796

16 1.628 + .037 39+6 63+11 137+15 200+25 0.570

\*cpm = cpm/larvae

# all values are mean + standard error of 12 replicate samples

+ No. of larvae used per sample: age 10-30 larvae, age 12-25 larvae ages
 14 and 16-15 larvae

Figure 1\* shows a typical response curve of CO 2 production in relation to incubation time. There was usually a three hour lag period, during which the initial ingestion, absorption and utilization of the labeled substrate takes place. After four hours the system generally equilibrates, and the turn-over rate of carbon can be estimated by the slope of the regression line, as shown on the graph.

The values for respiration rate and food intake shown in Table 1 were calculated from data taken during the 4, 5 and 6 hour incubations, when the system had achieved
an equilibrium.

Results obtained by measuring CO 2 production and food intake with the carbon-14 labeled glucose method have been compared with two other methods. CO 2 production and O 2 consumption were assessed using a Warburg Apparatus; and food intake was measured by a method using fecal uric acid to determine the proportion of uneaten food and faeces in a test diet (Bhattacharya and Waldbauer, 1970). In both cases the results of the C 14 method were highly correlated with those obtained with the other two methods.

Figure 1. A typical response curve of CO 2 production of Tribolium castaneum. Each data point represents the mean of four replicate samples at each time of
 incubation.

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Food analysis of Tribolium castaneum larvae

The idea of using insects and their larvae for human food is not as unusual as it may seem. According to Bodenheimer (1951), all tropical native populations include insects and their larvae as a staple in their diet. The food in these regions is greatly lacking in animal components and the insects serve to make up some of the deficiencies in the native’s diet. In fact, Bodenheimer states, the use of insects as food explains why many native populations are healthy in spite of their otherwise poor diet.

At the present time, we in America are beginning to experience a shortage of meat in our diet. Although available, the high price of meat places it out of reach for many people. It is possible that we will soon be searching for other sources of protein and fat in our diet. This study was made to discover if one of these sources can be the larvae of Tribolium castaneum.

Methods and procedures

The initial breeding beetles, Tribolium castaneum, were obtained from the Tribolium Stock Center. The beetles were placed in 3 pound coffee cans with a mixture of 98% unbleached flour and 2% brewers’ yeast. They were grown in an environmental room set at a constant temperature of 29 0 C and a constant humidity of 60%.

The beetles were checked every two to four days. The larvae were allowed to grow as large as possible for the experiment (about 1 month old). When a few pupae were seen among them, the larvae were removed from the flour by sifting through two separate screens; size 20 mesh for the beetles and size 28 mesh for the larvae.

The larvae were then killed by freezing and placed in a large flask. When the flask was full, the larvae were freeze-dried for one week and placed in a closed container with silica gel to prevent any moisture uptake or bacterial growth.

The moisture content of the freeze-dried larvae was determined by the direct heat method. For each determination, 5 grams of prepared sample were used.

To determine the ash content of the larvae, 2 or 3 grams of prepared sample were weighed out into a pyrex crucible. The sample was then charred in the crucible over an open Bunsen burner. Following charring, the samples were ashed at 560 0 C in a muffle furnace for three hours.

The ether extraction method was used for determining the fat content of the larvae. In each determination 10 grams of sample were used.

The method used to analyze the protein content of the larvae was the Kjeldahl-Wilfarth-Gunning method for determination of the total orgnic nitrogen. The equipment used for the analysis was a LabConCo micro-Kjeldahl Digestor and LabConCo Micro Distillation Apparatus. About 30 mg of pre-dried sample were used in each determination. The percentage of nitrogen in the sample was then calculated, using the formula suggested by Joslyn (1970)

 % w/w nitrogen =
 (ml of std. acid for sample-ml of std. acid for blank) XN X14.
 mg of sample
According to Jacobs (1970), the percentage protein is calculated by multiplying the percentage nitrogen by 6.25. This factor was used to determine the protein content.

The determination of the amino acids present in the larvae was carried out by descending paper chromatography. To free the amino acids from the larvae, two grams of prepared sample were weighed out and dissolved in 30 ml of 6N HC1. The mixture was then refluxed for 24 hours. After cooling, the sample was refrigerated during the preparation for the paper chromatography run. The above procedure was repeated with a second sample to insure the validity of the results.

The reading was accomplished by comparing the spots made by the amino acids in the sample solutions with the known amino acids in six previously prepared test solutions. The results obtained in the experiments are summarized in Table 1 and Table 2.

Discussion

The larvae of Tribolium castaneum can be considered protein-rich when compared to beef. Bodenheimer (1951) gives the protein value of beef as 16.9%. Jacobs (1970) also lists various protein values for beef and they range from 12-39%. Most values are less than the 28% found in the larvae. The larvae are higher in fat when compared to fresh beef. Fat values for fresh beef are 0.5%-9.52%. Moisture content in the freeze-dried larvae is in all cases lower than fresh or prepared beef which has from 48-80% moisture. The ash content of the larvae is higher than fresh beef which is usually about 1% ash. Prepared beef has a higher ash content in most cases with the values ranging from 1.3% to 11.2% ash.

In conclusion, the results of this study show that the larvae of Tribolium castaneum could be considered a worthwhile supplement to the human diet. They are relatively high in protein and fat and they can be raised on a low-cost budget. With the continuing rise in human population and widespread famine in some parts of the world, every possible source of nourishment should be explored. We believe that the larvae of Tribolium castaneum could eventually be utilized for this purpose.

 Table # 1

 % Moisture % Ash % Fat % N %Protein

Run 1 15.12 3.43 15.63 4.58 28.63
 2 15.04 2.90 16.40 4.20 26.25
 3 14.97 2.73 15,80 3.41 21.31
 4 15.11 3.76 5.32 33.25
 5 5.51 34.44

Aver-
age % 15.06 3.21 15.70 4.60 28.78

 Table # 2

 Amino Acids Present in Tribolium castaneum larvae

 Cystine Rf 11
 Lysine Rf 12
 Glutamine Rf 19
 Glycine Rf 25
 Hydroxyproline Rf 27
 Threonine Rf 30
 \*Homocitrulline Rf 31
 Proline Rf 38
 Tyrosine Rf 45
 Tryptophan Rf 50
 \*Methionine Rf 51
 Phenylalanine Rf 61
 Leucine and Isoleucine Rf 68

\*by inference

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The effects of prolonged exposure of Tribolium brevicornis to ether,
specifically in the areas of mortality and fecundity

Introduction

The purpose of this study was to analyze the effects of etherization on mortality and fecundity of T. brevicornis.

Materials and Methods

Approximately 425 female pupae were isolated and allowed to develop to adulthood. When the beetles were 7-14 days old and egg laying had become constant, the females were divided into eight groups, each containing fifty of them. To each group 50 males were added. The medium in each jar consisted of a presifted wheat and yeast mixture in a ratio of 95% wheat to 5% yeast.

The cultures were then placed in an incubator set at 32 0 C and 60% RH. At prescribed 24 hour intervals each of the cultures was examined and the flour sifted and eggs removed, counted and the mean per female calculated. These data were obtained over a 72 hour period.

The eight groups were then etherized with anesthesia grade ether (J. T. Baker Chemical Company) for the following time intervals:

 Group 1 …………………….. 2 minutes
 Group 2 …………………… 5 minutes
 Group 3 …………………….. 7 minutes
 Group 4…………………… ..10 minutes
 Group 5…………………….. 15 minutes
 Group 6 ……………………. 20 minutes
 Group 7 ……………………. 22 minutes
 Group 8 ……………………. 25 minutes

After sufficient time for revival had been given (24 hours) the number of dead in each group was counted and the per cent mortality determined.

Every 24 hours, the cultures were examined, the eggs were counted and the mean number of eggs per female determined. This process was carried out for 13 days, until all the females had returned to their normal egg laying schedules.

Discussion

As can be seen from the data on page 123 T. brevicornis females appear to lay approximately 3.38 eggs in a given 24 hour period, under conditions considered optimal for this species. (32 0 C and 60% RH). This figure is considerably lower than that previously determined for T. castaneum and T. confusum, which is an average of
15 eggs per day (Gray 1948, Park and Frank 1948).

Periods of etherization of less than five minutes appeared to have little effect on the fecundity of the beetles, and within a period of three days the females were back on their respective egg-laying schedules.

Time of exposure in excess of five minutes however produced rather dramatic effects on fecundity, with drastic decreases in the number of eggs laid per day. In general, the longer the time of exposure, to ether the more dramatic the initial effect on the daily egg laying of these organisms, and correspondingly the greater the period of time required for the females to return to a regular schedule.

The LD 50 of T.brevicornis appears to be about 25 minutes, these values are similar to those obtained by Sokoloff (1960 a, b) for T. castaneum.

I wish to express my thanks to Dr. A. Sokoloff for his advice and suggestions. (This project was funded by Army Grant RDRD LP 11790-LS.)

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Flying Behavior in Tribolium brevicornis

Introduction

This relatively brief experiment was conducted with the purpose of determining whether or not T.brevicornis has the capacity for flight as does T. castaneum or whether like T. confusum it lacks it.

Materials and Methods

A plastic bread box was employed in this experiment, as a flying chamber. Into the container were placed two petri dishes separated by a distance of ten inches. Into one of the dishes was placed 20 grams of WY medium and into the other were placed 200 Tribolium brevicornis (the dish containing them was thoroughly cleaned to prevent any organisms from climbing out).

A lid was placed over the box and it and its contents were placed in an incubator set at 32 0 C and 60% R.H. The study lasted five months and was repeated in triplicate. Periodically the box was examined and the flour sifted to see if any beetles had flown out of their place of confinement and landed in the flour or other areas in the plastic box.

Results and Discussion

In the period of five months, over which this experiment was conducted some 600 beetles were tested for flying ability. During that period not one beetle was observed to have flown, although several were seen attempting to.

It appears that Tribolium brevicornis lacks the capability of flight and must therefore disperse by crawling or with the help of man.

My thanks to Dr. A. Sokoloff for his help and suggestions. (This project was funded by Army Grant RDRD LP 11790-LS.)

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Effects of two moldy diets on Tribolium confusum

The potential danger to man and livestock of mycotoxin-producing fungi on stored-grain has been the subject of much recent research (Lynch, 1972; Mirocha and Christensen, 1974; Culvenor, 1974). The relationships between the fungi and insects that live in this habitat is not well known (Sinha, et al. 1969; Rao, et al. 1971; David and Mills. 1974; Harein and de las Casas, 1974).. Agrawal, et al. (1957) and Sikorowski (1964) have shown that insects disperse spores of storage molds from moldy areas to clean areas of stored grain. However, Sinha (1966, 1968) reported that T. confusum does not feed or reproduce on Aspergillus ochraceus Wilhelm when the mold is grown on agar. The following investigation was to determine the effects of two mycotoxin-producing fungi, grown on grain, on the biology of T. confusum.

Materials and Methods

A.ochraceus is known to produce several toxic metabolites, the most notable being ochratoxin A (Steyn, 1971; Applegate and Chipley, 1973). A high ochratoxin-A-producing strain (Isolate NRRL 3174) of A.ochraceus is very toxic to chicks (Doupnik and Peckman, 1970). Chaetomium globosum Kunze (Isolate WP-8) is known to be toxic to rats (Christensen, et al. 1966). An additional isolate of G. globosum (Isolate AC) has shown biological control properties against corn root infections (Kommedahl and Mew, 1975). It has no known toxicity to homoiotherms.

The 3 isolates were inoculated on twice-autoclaved mixtures of wheat and rice (1:1). Un-inoculated flasks (control) and inoculated flasks were cultured for 2 weeks at 27 0 C and 75% RH. The contents of the flasks were then allowed to air dry (21 0 C) for 4 days before being ground to a particle size that would pass through a #40 standard grain sieve. Test diets were made up with 5% brewers’ yeast and various concentrations of the inoculated wheat: rice mixture. Fifty unsexed 2-week-old adults of T. confusum were added to containers were kept at 27 0 C and 75% for 6 weeks after which the original adults were removed and weighed, mortality determined, and progeny counted. The insect media were then mixed with a rate ration (1:1) and fed ad libitum to 21-day-old female rats for 7 days. Two rats were used at each treatment level.

Results

There were no significant differences in mortality or weight of the parent beetles. However, the number of progeny produced was significantly different for the different concentrations of the moldy diet. With 30% C. globosum (WP-8) in the diet there were more progeny than at any other concentration. Increasing concentrations of C..globosum (AC) resulted in a linear increase in number of progeny. A.ochraceus (NRRL 3174) quite dramatically increased the number of progeny produced; 3 to 4 times more than at lower concentrations. The results are summarized in Figure 1.

In the rat trials, C. globosum (AC) resulted in rats gaining less weight (24.5 g avg.) than the control treatment (38.7 g avg.). Isolate WP-8 of C. globosum had no effect on the rats, except for the death of one following 7 days on the 100% insect diet. However, the 2nd rat on the same diet showed no ill effects. The rats fed A. ochraceus died after 3 to 5 days. Most exhibited extreme anorexia, although the rats on the 30% insect diet or less did do some feeding.

Discussion

It is apparent that A.ochraceus and C. globosum, as represented by the isolates fested, are not harmful to T. confusum, and could be considered beneficial. Yet, the same diet from moldy grain, represents a potential danger to homoiotherms, especially in the ase of A. ochraceus. Certainly, the presence of molds or insects in grain or cereal products destined for food or feed is not desirable and will result in a loss of quality and value. But the presence of both may represent a compounded problem; the increased spread of the mold, the increased number of insects, and the resulting lower quality of the item, even to the point of being a health hazard. (Work supported by a grant from The Gargill Foundation).

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Natural infestation of stored rapeseed bulk

As part of a long-term project to study the storage stability of various commercially grown crops in Western Canada, 45.72 metric tons of locally grown Zephyr rapeseed with about 10% dockage were stored in August, 1973 at 7-9% moisture content and 29 to 35 0 C in a new circular metal bin (557 cm in diameter, 4000 bu capacity) in Glenlea, Manitoba. Temperature measurements and samples were taken at the center, periphery and other locations of the rapeseed bulk at 0-, 100-, 200-, and 270-cm distances from the floor. Fourteen samples were collected with a 300 cc (177 g) torpedo probe. The variables measured were: temperature, moisture, fat acidity, seed germination, microflora, insects, and mites. Preliminary results for 1974 are as follows. Temperature ranged from -14 to 7 0 C in February and 14 to 22 0 C in September. Except for one or two locatios, seed moisture ontent remained between 7.5 and 9.2%. About 12 months after initial storage the entire bulk was heavily infested with two species of mites, Acarus immobilis Griffiths (max. no. per sample, 2050) and its predator Blattisocius keegani (Fox) (max. no. per sample, 65) and psocids; light infestation of Cheylelus eruditus (Schr.), Glycyphagus destructor (Schr.), and the rusty grain beetle, Cryptolestus ferrugineus (Steph.) also occurred.

The moisture content of the rapeseed increased to about 12% along a column about one-half meter inside the south-west wall, upto 200 cm from the floor level. This zone was heavily infeted with fungi such as Penicillium spp.(72%), Aspergillus versicolor (76%), and A. ochraceus (12%); it also showed the highest level of infestation by mites and insects. Abnormal heating did not occur anywhere in the rapeseed bulk.

Fat acidity (mg/g) of the rapeseed, which had a 43-45% oil content, ranged from 7.35 to 8.88 in most samples tested so far. In one spot along the affected moldy area along the wall, however, fat acidity rose to 30.67 mg.

These preliminary results suggest that large bulks of rapeseed stored in metal bins are highly succeptible to infestation of stored-product mites, such as A. immobilis within a year after storage. Heavy fungal and arthropod infestations may also occur in pockets near the south wall where grain temperatures are often higher than in other parts of the bin.

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Differences in fecundity and egg cannibalism of T. audax and T. madens

Sokoloff (1973) found that, when T. audax and T. madens are reared together in different media, T. madens eliminates T. audax regardless of the medium in which they are reared. He also reported that at 32 0 and 70% relative humidity T. audax requires about five days longer than T. madens to complete its life cycle (i.e. 38 days are required for the latter and 43 days for T. audax). Furthermore, T. audax has a pupal period of 14 days and T. madens only 10 days, hence the latter is far less vulnerable to larval or adult predation at this stage. Also, the onset of maturity occurs at 40 days after the eggs of T. madens are laid while for T. audax the onset of maturity occurs at 50th day. T. audax was able to persist longer when it was reared together with T. audax when the rearing medium was wheat plus yeast rather corn or corn plus yeast.

The purpose of this experiment was to obtain further data on the differences in fecundity and/or egg cannibalism of these two species in two different media.

Method

Egg farms of the two species were established in medium turned red due to the presence of neutral red dye. When eggs are laid they are usually moist and they pick up particles of dyed material, so that these eggs are distinguishable, when placed in white flour medium from recently laid eggs. For each species, fifty marked eggs were introduced for a 24 hour period into each of 10 vials containing 25 pairs of adults, At the end of 24 hours, the white and red eggs were counted. The red eggs were returned to the experimental vial and the experiment continued for another 24 hour period. The experiment was terminated after counting the dyed and non-dyed eggs.

Results

The data are shown in Tables 1 and 2. Table 1 shows the numbers of eggs surviving the first and second 24 hours as well as the total 48 hour period. The analyses of the data in Table 1 lead to the conclusion that the two species are different in the following ways:

1. Cannibalism
2. T.audax males are less cannibalistic than females in whole wheat flour plus yeast, but about equally cannibalistic in corn. For T. madens cannibalism is low and there is no significant difference between the two sexes in wheat plus yeast. T. madens females only become more cannibalistic in corn than in whole wheat plus yeast.
3. The difference in egg cannibalism in the two species is not significant for males in wheat plus yeast but significant in corn, T. audax exceeding T. madens in egg elimination. For females, T. audax is not significantly different from T. madens in the first 24 hour period in wheat plus yeast but it is significantly cannibalistic for the second 24 hour period or for the total period. T. audax females again exceed T. madens in cannibalism in corn.
4. Fecundity
5. The fecundity of T. audax greatly exceeds that of T. madens fecundity of
T.audax is three to five times greater than of T. madens.
6. Fecundity of the two species is constant and characteristic: The values of the first 24 hours do not differ from the values of the next 24 hours.
7. Fecundity of T.audax is greatly affected by the medium used: it is much greater in wheat plus yeast and much less in corn. The low fecundity of T. madens is not significantly affected by the two media used.

Conclusion

From the above results and those given previously by Sokoloff (1973) it seems evident that in these experiments it is the differences in the period of development that are important in eliminating T. audax when this species is introduced with T. madens into the same environment.

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Acknowledgment

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 Table 1

Mean numbers of eggs surviving egg cannibalism when exposed to adult Tribolium
audax and Tribolium madens in two different media for two 24 hour periods

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 T. audax

Predator Period N Wheat N Corn
Sex m + S.E. m + S.E.

Males 1st 24 hrs. 10 49.6 + 0.2 10 43.6 + 1.2

 2nd 24 hrs. 10 47.5 + 0.85 10 39.5 + 1.45

 48 hrs. 20 48.6 + 0.48 20 41.5 + 1.03

Females 1st 24 hrs. 10 43.2 + 1.8 10 40.8 + 0.95

 2nd 24 hrs. 10 37.8 + 2.6 10 34.1 + 1.5

 48 hrs. 20 40.5 + 1.66 20 37.5 + 1.16

 T. madens

Males 1st 24 hrs. 10 48.3 + 0.54 10 49.6 + 0.2

 2nd 24 hrs. 10 46.9 + 0.5 10 48.1 + 0.9

 48 hrs. 20 47.6 + 0.51 20 48.8 + 0.48

Females 1st 24 hrs. 10 47.5 + 0.6 10 47.2 + 0.8

 2nd 24 hrs. 10 45.8 + 0.6 10 43.6 + 1.3

 48 hrs. 20 46.6 + 0.45 20 45.4 + 0.86

 Table 2

Fecundity of Tl audax and T. madens in two successive 24 hour periods in wheat plus yeast and in corn.

 T. audax

 Wheat plus Yeast Corn
 N M + S.E. N m + S.E.

 10 63.3 + 7.66 10 48.5 + 2.32

 10 71,1 + 7.57 10 47.6 + 3.16

 T. madens

 Wheat plus Yeast Corn
 N m + S. E. N m + S. E.

 10 19.5 + 5.12 10 16.9 + 4,17

 10 13.1 + 3.09 10 15.5 + 4.09

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Some behavioral observations on various species of Tribolium

Five species of Tribolium, (anaphe, audax, brevicornis, destrictor and maden), were starved for one day at room temperature and their behavioral dispersion and activity, before and after the addition of a standard medium placed in the centre of a plastic observation box (14 x 10 cm and 4 cm high) are recorded in the following Table.

Species Total Dispersion Activity in relation to the
 number No. of No. of addition of food
 observed individuals insects Before After
 in groups 3 hrs. 6 hrs
 (no. of insects
 outside medium)

audax 101 5 16 - mainly quiet none none
 27 - individuals
 53 move from one
 group to another

anaphe 81 all individually none - quiet (nearly all most
 immobile)
 - more quinine
 secreted

destructor 76 3 19 - mainly quiet 38 27
 21 } one
 1 } com-
 32 } plex
 clumps of 3 layers

brevicornis 60 34 15 - active 3 1
 11 (1 pair copulating)

madens 42 6 5 - mainly quiet 23 11
 8
 23

Immediately after the addition of food T.brevicornis became more active and oriented itself toward the food.

T. audax seemed to have a low cannibalistic tendency. Among 1718 adults, 3 weeks old, there were no cannibalized adults, pupae or larvae even though this large number was maintained in 117 grams of standard medium (1 g/15 adults). At that density there was no detectable quinine odour. The presence of only few pupae and larvae indicate that population size may be regulated by the abioity of females to stop laying eggs at high adult density or that adults may only cannibalize the eggs or early larvbae.

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Survival of T. audax on corn and wheat flour with or without yeast

Sokoloff et al. (1974, TIB 17: 73) demonstrated that adults of a laboratory strain of T.audax, when given a choice between corn flour or wheat flour (both supplemented with yeast), preferred to go to the corn flour and laid more eggs on corn rather than on wheat. This note is on the survival of the same strain of this species on the two kinds of media with or without yeast. For each of the four experimental units 100 unsexed adults less than 21 days old were placed in 5 grams of a specific medium and kept at
a constant temperature of 33 0 C and 65% RH. The insects were observed weekly for
a period of 70 days. From the following table it is clear that the corn flour provides a more suitable medium than the wheat flour for the survival of this species regardless of the presence of yeast. However, the addition of yeast to the wheat flour has increased the number of insects that survived to the end of the observation period thus indicating the significance of the yeast at the sub-optimal nutritional levels.

The age effect may below in the “environmental” class of factors. If some substance is contributed by the female to the egg, it is possible that in older females the quantity of this substance is reduced. A similar hypothesis for parent age effect on offspring developmental period of T. confusum was offered by Raychaudhuri and Butz (1965). In parents with a + phenotype, which produce fewer eu offspring, the effect of such a contribution may be more noticeable than in parents with the mutant phenotype.

TABLE 1. Classification results of the offspring in different crosses.

1. Parents with normal phenotype (+)

 Offspring
 phenotype: + eu X 2 (ldf) % eu

 OF x OM 97 58 } 10.60\*\* 37.4
 YF x YM 67 85 } 55.9

 OF x YM 221 137} 15.07\*\* 38.3
 YF x OM 202 220} 49.8

1. Parents with mutant phenotype (eu)

 OF x OM 72 74} 5.62\* 50.7
 YF x YM 45 83} 64.8

 OF x YM 149 159} 3.55 ns 51.6
 YF x OM 154 220} 58.8

\*\* P 0.01

\*P 0.05

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Preliminary study of the effects of altosid R, a juvenile hormone analog, on the adult emergency of Tribolium castaneum and Tribolium confusum

Introduction

The purpose of this research project is to determine the effect which varying concentrations of the juvenile hormone analog, isopropyl 11-methoxy-e, 7, 11-trimethyl-2, 4-dodecadienoate (Altosid R), has on the morphogenetic development of two species of flour beetles, Tribolium castaneum and T. confusum, when mixed in their nutrient-growth medium.

The experimental techniques described herein were designed to determine the general ranges of effective dosages for each of these species. More precise dosage-effect data will be given in a later paper.

Altosid R has been shown to be a highly effective morphogenetic control agent against holometabolous insects (Henrich, Staal, and Siddall, 1973; Cerf and Georghiou, 1972), and thus was chosen for testing in this experiment as a possible new means for controlling these two species of flour beetles.

Methods and materials

In this experiment carried out as an Independent Study Project under Dr. A.Sokoloff, two species of flour beetles, Tribolium castaneum and T. confusum, were tested for changes in their morphogenetic development when exposed to the juvenile hormone analog, isopropyl 11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate (Altosid R, Zoecon Corp.). The original stocks of beetles, which were supplied by the Tribolium Stock Center at the California State College in San Bernardino, were not selected for any resistance to insecticides or juvenile hormone analogs. In addition, all of the untreated growth medium (wheat flour: yeast, 19:1 2/2) was supplied by the same stock center.

Throughout the duration of the experiment, exposure of the beetles to be juvenile hormone was accomplished by rearing them in medium trated with the compound. This treatment consisted of thoroughly mixing appropriate acetone dilutions of Altosid R into the medium with controls being; medium treated with acetone only, and no treatment at all. The Altosid R concentrations tested in the growth medium were: 10.0, 5.0, 2.0, 1.0, 0.5, 0.1, 0.03, 0.01, 0.001 and 0.0001 parts per million (ppm).

Three replicates at each concentration and for each control were re, each being started on a different day. Ten adult beetles, chosen at random from stock cultures, were introduced into each rearing jar at the beginning of the experiment. After five weeks, the contents of each rearing jar were run through a sieve, and the number of successfully emerged adults were counted. Partially emerged or dead adults were not counted.

Results and discussion

The data obtained in this experiment show that Altosid R exerts a very definite morphogenetic effect on both species tested. Tribolium castaneum is more affected by the compound than T. confusum: adult emergence of the former species is completely depressed at concentrations down to 0.1 ppm, and adult emergence of the latter is completely depressed at concentrations between one and two parts per million.

These data agree with those presented in a screening experiment conducted by Strong and Kiekman (1973), who stated that both of these species of flour beetles were very strongly affected by this compound at dosages down to five parts per million (the lowest concentration they tested).

The preliminary data obtained thus far show that Altosid R can be used as an effective control agent against both of the species of flour beetles tested in this study. The ease with which it can be applied to either stored grain or flour, and the low effective dosages required, make it a relatively attractive compound for this purpose.

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Survival and developmental period of two T.castaneum strains at 25 0 C.

The results of another experiment (to be reported elsewhere), made it necessary to compare survival and developmental period of two T. castaneum strains at 25 0 C.

Materials and methods

The two experimental strains were our stock wild (++), and Black (bb) strains of T.castaneum (see stock list).

Eggs for each strain were obtained from an egg farm, in which about 300 adults oviposited for 3 days at 30 0C. Samples of 100 eggs (single strain and mixed) were introduced into vials with 1 or 5 gr of standard medium (flour and brewers’ yeast in the ratio 100:5), producing two input egg densities: 100/g and 20/g.

The mixed cultures (M) contained 50 eggs of each strain. The final number of replicates was as follows (3 replicates were lost to disease).

 Single Strain Mixed

 ++ bb M
 5g 10 10 10

 1g` 10 8 9

The vials were maintained at 25 + 1 0 C (humidity not controlled but close to 70%). Beginning on day 25, the vials were sifted in 2-4 day intervals and pupae removed, separated by sex, counted and, in M, held for classification by color as adults. In bb and ++, the pupae were discarded after counting.

Calculations.

Parameters calculated for each vial included: Survival (number of pupae produced from the input-100 eggs), and developmental period (median number of days, egg to pupa. Day 0 was the set up date of the egg farms). In M, the proportion of bb per vial was also determined.

Results

1. Survival. The survival to pupation in the experimental replicates is summarized in Table 1.

 TABLE 1

 Percent Survival (egg to Pupa)

 1g 5g

 n Y SE n Y SE

 ++ 10 42.3 4.33 10 66.5 4.59

 bb 8 34.9 5.68 10 62.4 4.43

 M 9 29.8 3.81 10 68.8 3.12

In all three population types, survival was significantly lower in the 1 g compared to 5 g vials. (P 0.001 by t-tests, with unequal variances, Sokal and Rohlf, 1969). When all 3 population types were compared by Analysis of Variance within each input density, no significant differences in survival were found.

In our stock strains, which are periodically assayed for several parameters in 5 g vials at 30 0 C (our standard rearing temperature) average survival to pupation ranged from 82-88% in ++ and 75-85% in bb. Values obtained in the present experiment in 5 g vials are considerably lower.

Survival of pupae to adulthood in M was 92.5% in 5 g vials, but only 52% in 1 g. (Pooled data from all replicates: 639 adults from 691 pupae in 5 g, 142 adults from 273 pupae in 1 g). The difference is highly significant (P 0.001 by a test of percentages, Sokal and Rohlf, 1969).

II Developmental period The average median number of days from egg to pupa in the experimental combinations are given in Table 2.

TABLE 2

Developmental period (days, egg to pupa).

 1 g 5 g

 replicates (n) Y SE n Y SE

++ 10 48.0 1.13 10 41.0 0.27

bb 8 40.5 1.35 10 33.3 0.13

M 9 43.4 0.96 10 37.0 0.38

(Paired-comparison t tests have indicated no difference in developmental period between the sexes within vials). It is clear that development was delayed by about 7 days in the 1 g compared to 5 g vials (the difference was significant in ++ and bb, but not in M). Within each density, development was slowest in ++, fastest in bb, and M was intermediate. These differences were significant at least at the 5% level. Figure 1 illustrates the cumulative percent pupation in the 6 experimental combinations. In our stock strains average median developmental time to pupation in 5 g vials at 30 0 C ranges between 22-25 days in ++, 21-24 days in bb. Development in the present experiment (at 25 0 C) was very much longer, in particular in ++.

III Composition of the mixed populations. The results of adult classification in the M vials were as follows: (all replicates pooled).

 ++ bb total %bb

 5 g 300 339 639 53.0

 1 g 56 86 142 60.6

Apparently, more bb than ++ emerged from the M vials. The deviation from the 1:1 expectation was significant in the 1 g (X 2 = 6.3, 1 df), but not in the 5 g.

Median developmental period of ++ and bb in M vials was calculated only for those organisms which reached the adult stage. The medians show the same relationship as in single-strain vials (number of pupae in parenthesis):

 ++ bb

 5 g 40.6 (300) 33.4 (339)

 1 g 43/7 (56) 37.0 (86)

Clearly, in both densities, ++ developed more slowly than bb in the same M vials.

The frequency distribution of pupation in the M vials at both densities I illustrated in Figure 2. The frequency distribution is bimodal at both densities, with peaks at day 34 and 40-43 in 5 g, and at day 34 and 45-48 in 1 g.

At the top of figure 2 we illustrate the proportion of bb individuals in each census date of the M vials. The early samples contained almost entirely bb, while late samples contained mainly ++. The following table presents the number of individuals of both strains in M prior and after day 36 (this day was shosen arbitrarily at the drop between the two peaks).

 5 g 1 g

 ++ bb total ++ bb total

up to day 36 18 222 240 1 40 41

after day 36 282 117 399 55 46 101
 639 142

G-tests of independence showed that the distributions of ++ and bb in the two peaks were highly significantly different at both densities (P 0.001).

Discussion

Little information is available on Tribolium survival and development at low temperatures, since in most laboratories the insect is reared at temperatures close to 30 0C. Mikulski (1936 a, b) presents data showing that at a constant 25 0 C temperature, egg survival of T. confusum was as low as 45% and pupal survival, 62% as compared with 100% and 80% respectively at 30 0 C, and pupal period is increased. Howe (1956) presents a table from which it can be learnt that survival of T. castaneum at 25 0C and 30 0C was the same, but developmental time was prolonged at 25 0C.

In the present experiment, survival (at 25 0C) was clearly lower than is commonly found in our stock strains (at 30 0C), when measured at the low density, of 100 eggs in 5 g of medium. Survival in the 1 g vials was considerably lower than in 5 g. The negative effect of increased density on survival in T. castaneum strains of the same origin had been reported (Sokal and Karten, 1964).

The most interesting aspect of the results is the differential effect of the lowered temperature on the two strains. Clearly, developmental rate in ++ was considerably more delayed than that of bb compared to the stock at 30 0C. It is interesting that no differences in survival were found between the strains at either density: the effect on development was independent of survival.

The relationship of ++, bb and M developmental times at both densities was ++ M bb, as can be expected if no interaction occurs between the strains in mixed cultures.

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NOTE – Caption for Figure 2 (page 146) should read:

 Frequency polygon of pupation of M at the two densities, and proportion of
 bb individuals at each census time (bars).
 5 g: solid lines, Right-hand bars.
 1 g: broken lines, Left-hand bars.

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The effect of T-2 toxin on Tribolium confusum: growth, molting, and 0 2 consumption

T-2 toxin is one of several metabolites of Fusarium tricinctum, a mold commonly found in stored grain in northern temperate regions. It is known to be cytotosic, to inhibit DNA and protein synthesis, and to induce strong dermal raactions when applied to the skin of animals. In rats it has an oral LD 50 of 3.8 mg/kg (Bamburg, 1972). We have tested T-2 toxin for insecticidal activity on Tribolium confusum and found its effects to be mostly sub-lethal (Wright et al., 1973).

Newly hatched T.confusum larvae were exposed to 100 ppm T-2 toxin in a medium of whole wheat flour + 5% yeast. One group of these larvae (4 vials containing 15 larvae each) were weighed every fourth day on a rotational basis to avoid handling effects. In a second group, 10 individuals were observed for ecdysis. Each larva was placed in a small plastic cube with a film of flour on the bottom. Observations were made daily without handling the larvae. Exuviae were removed. A third group (20 larvae/clask) was placed in a Gilson respirometer and 0 2 consumption recorded.

Figure 1 depicts the growth of larvae by increase in weight over a 22 day period. Control larvae were heavier from the first day. The rate of growth accelerated slowly the first 10 days followed by an increased but steady growth rate is the last 10 days. It seems that the treated larvae followed the pattern of the control but grew more slowly. This was not exactly true. Table 1 shows the growth as a ratio of the weight at one molt to that of the previous molt as described by Howe (1968). Both the control and the treated larvae doubled their weight during the second instar. Remember that they did not weigh the same. The treated larvae dropped further behind in the third instar. However, the fourth instar quadrupled its own weight in an effort to catch up. This effort was not enough. Before pupation could take place, an extra or sixth molt was required. The final instar then made up the necessary weight.

Larvae treated with T-2 toxin consumed less 0 2 than the controls. The effect was noticeable in the first 24 hours. By the second and third day there was 24% difference from the control. The lower 0 2 consumption illustrates a physiological stress resulting from exposure to this mycotoxin. Although T-2 toxin is a fungal metabolite with high activity in mammals, it had no extreme effect on the confused flour beetle.

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Table 1. The Ratio of the Weight of Larval T. confusum at one Ecdysis to that of the
 Previous Ecdysis.

 Ecdysis Control T-2 toxin

 1 - -

 2 2.0 2.0

 3 3.0 2.5

 4 3.0 4.0

 5 3.0 3.0

 6 - 1.5

Table 2. Total Oxygen Consumed by Larval T. confusum from Hatch to 3 days.

Hours from ul 0 2 consumed/mg live weight
 Hatch
 Control T-2 toxin

 24 55 45

 48 215 165

 72 620 480

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The effect of inbreeding on egg laying of T. castaneum

Inbreeding was carried out in two non-contemporary sets of 50 lines each, all of them established by brother x sister single pair matings and continued for four generations (F4 = 0.594). The number of eggs laid from the 7th to the 9th day after adult emergence was evaluated from two samples of five virgin female off-spring each per line and generation. All individuals were kept at 33 0C except for the egg laying test, during which one of the samples was scored at 28 0C and the other at 33 0C. In each set of lines a control line was reproduced every generation by 50 single pair matings, each contributing one offspring of either sex to be mated at random avoiding sib matings (F4=0.01). From each of these matings two samples of five virgin female offspring were taken and each one scored at one of the two temperatures every generation. The initial base population was sampled from the Consejo population (OROZCO & BELL, 1974a) maintained in this laboratory at 33 0C since 1964.

The parameters of the base population estimated for the two temperatures are shown in Table 1. Average egg laying was significantly higher (P 0.01) at 33 0C.

Table 1

 Parameters of the base population

 Temperature

 28 0C 33 0C

 Mean 5.06 8.23

 Standard error 0.10 0.18

 Phenotypic variance 21.10 54.32

 h 2 + s.e. (h 2) 0.16+0.08 0.14+0.08

Table 2 shows the evolution of the mean egg laying (X) averaged over the inbred lines (I) and that of the control lines ©, as well as the estimated inbreeding depression D = X I – X C, over the four generation period considered, for the pool of the two sets of lines. Mean egg number at 28 0C and 33 0C in both the average of the inbred and in the control lines, plotted against generation number, is shown in Figure 1 both for the two sets of lines and for the pooled data. No significant inbreeding depression was detected in any generation either at 28 0C or 33 0C. The two significant differences between the mean of the inbred and the mean of the control lines were found in generations 3 and 4 at 28 0C and they were a case of “inbreeding enhancement” rather than inbreeding depression.

A large number of inbred lines were lost and by the end of the experiment, only 12 lines in set I and 15 lines in set II remained.

The absence of inbreeding depression suggests that the loci controlling egg laying, act largely in an additive manner at both temperatures, although additive x additive epistasis may be present. Loci showing dominance gene action might also be present but the gene frequencies of these loci in the base population should be low enough not to be detected by inbreeding, although these frequencies may be increased by selection (OROZCO & BELL, 1974b). Of course the validity of our interpretation rests on the assumption of neutrality or quasineutrality of the loci involved and a large number of lines were lost as inbreeding progressed. Nevertheless, as far as the surviving lines are concerned the main conclusion still holds and egg laying of virgin females in the Consejo base population could then in principle be considered a peripheral trait with respect to fitness.

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Methods of studying the abdominal musculature of Tribolium and other beetles

The Morphology of the abdominal musculature of the adult beetles can be studied either in dissection of fresh or previously fixed and preserved material or in cleared permanent preparations of the same material.

Dissections

Live specimens are dissected directly in 50% or 70% alcohol to gradually dissolve the fat bodies which otherwise obscure the view during dissection. The specimens preserved in alcohol may either be dissected in the same alcohol or if they have become brittle due to long preservation, they may be transferred into a less strong alcohol or to water to soften the muscles. Before dissection, the elytra and wings of the insect are removed , the legs are straightened by pulling them apart and fixing them in the dissection dish with pins placed horizontally over the legs (the dissection dish is made by filling a small petri-dish with wax or plasticine).

The dissection is made in one of two methods:

The tergum of the abdomen and metathorax is lifted up by cutting up the pleural region of the body; this method is useful in studying the details of the tergal and sterna muscles. The metatergum must be kept with the tergum of the abdomen to see the anterior attachments of the muscles of tergite 1, which arise from the antecosta of tergite 2 and insert upon the metapostnotum passing over tergite 1.

Otherwise, the whole body may be sagittally cut by one or in larger insects by two paramedian sagittal cuts; this method is very useful in studying the full details of the pleural and tergosternal muscles.

After such cuttings the other body contents are removed and the musculature can be studied in this state under a binocular stereo microscope with reflected lighting. Studying the musculature in this state is particularly useful when examining species with a dark or hairy abdomen, especially in the latter. For example, when studying the abdomen of some bruchids, etc., it is very difficult to see the muscles in the cleared preparations described below. The muscles of such beetles are nearly invisible as they become more transparent during the clearing process and are also obscured by the interference of the hairs which give a linear appearance in transmitted light.

Permanent Preparations

The above dissections may be mounted for permanent use. This method is very useful when studying insects with translucent abdominal sclerites. The species with dark abdominal sclerites should be bleached in commercial hydrogen peroxide (20 vols.) before or after dissection to use in permanent preparations. This bleaching process is very critical for freshly dissected insects because the already white and fresh muscles are easily dissolved or over-bleached, in which case they do not retain the stain and remain too transparent. This bleaching process does not affect the muscles of the specimen preserved in alcohol because during preservation the muscles are hardened and become amber coloured. This colour is not removed in bleaching, and the muscles are easier to stain.

The following procedure is used in making permanent preparations from the dissections described above.

1. If the specimens are already fixed and preserved, transfer the dissections into 70% alcohol for 30 minutes. If not, fix the dissections in Bouin’s fluid for an hour, wash under tap water and then transfer into 70% alcohol for 30 minutes.
2. Stain in Borax-Carmine for a few hours.
3. Wash off the excess stain with 70% alcohol, into which a few drops of HC1 may be added to differentiate the stain.
4. Immerse in 90% alcohol for 10 to 15 minutes.
5. Counterstain with Picro-Indigo-Carmine (See Eltringham, H., at the Claredon Press, Oxford) for 5 minutes (this time may be varied according to the size of the specimens to be stained, the small specimens stain faster), then wash off the excess stain in 90% alcohol.
6. Differentiate in 90% alcohol containing a few drops of acetic acid.
7. Transfer the specimens into absolute alcohol for an hour or more.
8. Immerse in Cove-oil until the fat bodies dissolve and the muscles are cleared , then mount in Canada-balsam.

 Notes – Technical

I found the specimens fixed in K.A.A.D. or C.F.A.A. and preserved in 70% alcohol, probably not more than a year, very satisfactory in studying the abdominal musculature. For these specimens staining is not really necessary. With fresh specimens, although it is possible to fix them in Bouin’s fluid in a short time, the muscles tend to become contracted and distorted in the fixative and very easily detached from the sclerites. Therefore they should always be stained to be seen well. In any case, where the staining is necessary, one of the staining steps may be omitted, as either of them alone
 is often satisfactory.

The same methods can also be employed in the morphological study of the abdominal musculature of the larva and pupa of the beetles.

I wish to thank my supervisor Dr. R.A. Crowson for his helpful suggestions and corrections.

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Effects of sevin on the mortality of Tribolium confusum

Introduction

Numerous studies have been performed on the effects of pesticides on flour beetles. Reported here is a simple experiment which can be used to demonstrate the establishment of an LD 50, the effect of a pesticide on reproduction, and pesticides as agents of selection.

Methods

Tribolium confusum were cultured in 95% unbleached whole wheat flour plus 5% yeast at 29 0C and 30% relative humidity. Experimental and control cultures consisted of 30 adult T.confusum in 50 g of medium in a 225 ml. glass jar. Three replicates each were established for the control and three experimental. Experimental cultures contained 1$, 5% or 10% powdered Sevin (carbaryl) by weight. The pesticide was mixed evenly into the flour and yeast medium. Dead beetles were removed at each census and only live beetles were counted.

Results

By the end of 24 hours, distinct differences existed among the cultures containing differing concentrations of Sevin (Table 1).

 Table 1. Survival of T. confusum at 24 hours. Data are number of beetles + one
 standard deviation.

Jar Control 1% Sevin 5% Sevin 10% Sevin

1 30 24 22 15
2 30 27 21 17
3 30 26 24 20

Total 90 77 67 52
 X 30 25.7 + 1.53 22.3 + 1.53 17.3 + 2.52

After 24 hours, all beetles were removed from the pesticide containing medium and placed in 50 grams of normal medium. All beetles exposed at each pesticide concentration were combined. Many surviving beetles appeared unable to walk or remain upright. Some were on their backs flexing their appendages. Disabled beetles were not censused but appeared to be much less abundant from the 1% Sevin exposure compared with populations exposed to 5% and 10% Sevin.

Mortality rate among the surviving beetles was monitored for 30 days following exposure to Sevin. During this time period, the beetle population exposed to 10% Sevin decreased 54%, while the surviving population exposed to 5% Sevin decreased by 27% and that exp9sed to 1% Sevin decreased by 13% (Table 2).

 Table 2. Mortality of T. confusum following 24 hour exposure to Sevin.

 Control 10% Sevin 5% Sevin 1% Sevin

Day 1 90 52 67 77
Day 4 90 34 57 71
Day 6 90 26 52 70
Day 15 87 25 50 68
Day 30 85 24 49 67

It appears that a 5% concentration of Sevin (bt weight) results in very nearly an Ld 50 in T. confusum under the conditions of this experiment. From Day 1 to Day 15 a total reduction from 90 to 50 beetles occurred (55.6% decrease). When the Day 15 data (Table 2) are plotted, number of survivors (Y) against concentration of pesticide (X), the result is a totally linear curve (Y = 9.480 + 4.787X) with a correlation coefficient of - .99. From this curve, a 6% concentration of Sevin would produce an LD 50 by Day 15.

On Day 30, the number of larvae produced by the beetle populations exposed to each of the 3 pesticide concentrations were censused. The results (Table 3) indicate that larval production was a function of starting density. It appears that exposure to Sevin did not adversely affect productivity although this cannot be said with certainty since larval production in control populations at similar densities were not censused.

No selection studies were included in this experiment although it is clear that the surviving beetles and their offspring could easily be studied for increased resistance to Sevin.

Table 3. Production of larvae at Day 30.

Conc. # Starting Adults # Larvae # Larvae/Starting Adult

10% 52 232 4,46
 5% 67 208 3.10
 1% 77 48 0.62

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Density, temperature and moisture variables and their influence on the development and survivorship of mealworms (Tenebrio) – A teaching experiment

Introduction

This experiment was designed for Population Biology laboratories of 24 students each which meet once a week for three hours. This design may be used successfully for labs with students numbering from 16 to 30. Below 16 students per laboratory there is too much work for each team. Only one of the two series described below is recommended for labs with the lower number of students. Approximate lab time required is two hours the first day, 1 ½ hours the second and one hour each for the third, fourth and fifth days.

Experiment – A study of 24 mealworm populations

Purpose: The objective of this experiment is to determine the possible impact of density (crowding), temperature and moisture on the development of the members of 24 mealworm populations.

Materials and methods:

1. Six student teams will be formed in each laboratory.
2. Each of the six teams will be given: four 8 oz. jars, 400 ml. bran (100 ml. for each jar), eight paper discs each week (two for each jar, moistened with distilled water), four 2-ply pieces of gauze (to cover each jar), four rubber bands (to attach gauze to the jar), four millimeter rulers (to measure mealworm lenths) and 160 mealworms to be divided into four groups (4, 12, 36 and 108).
3. Two series (three teams each) will be established for each laboratory; series one will not use potato in the populations while series two will add 20 grams of fresh potato each week after removing the remains of the potato from the previous week.
4. Each team will work with four populations of mealworms numbering 4, 12, 36 and 108 mealworms respectively.
5. Each team in each of the two series will be assigned one of three specific growth temperatures for its four populations of mealworms; i.e., 50 0F., or Rm. Temp. (about 72 0F.), or 82 0F. There will then be a potato and no potato group for each of the three temperatures.
6. BEFORE adding the mealworm populations to the jars each team must collect data as indicated below for the first day.
7. AFTER collecting data assemble the populations as illustrated below:
8. Label each jar with the number of mealworms, growth temperature, presence or absence of potato, date names of team members and laboratory section number.
9. Place each set of populations in its assigned growth chamber (temperature) until next week.

Data:

First Day (week one) – This first day will require about two hours of orientation and sampling time. Before adding the mealworms to the bran in the jars, each team must weigh each mealworm population separately and record the weight on the data sheet. Each team must measure the length of each and every mealworm in each of the four populations. Record the length on the data sheet as the total length for each population in millimeters. From the total weights as well as lengths the averages can be calculated for each of the populations ad recorded on the data sheet. On your data sheet is for the TOTAL and X on your data sheet is for the average (mean). See Master Data Sheet. Students are responsible for all of the data taken in their laboratory section. Teams must record data neatly and clearly. Use black ink for the data sheet which will be used for laboratory data exchange at the end of the experiment. Ditto masters can be made only from good clear copies. Each student in the laboratory will receive
a copy of all the data included on the master data sheets (six per lab.).

Second Sampling Day (week two) – Each team should take about 1 ½ hours for the sampling of the populations. Taking one population at a time, each team must sort out the mealworm larvae, pupae and adults if any. Shake the bran through a screen fine enough to hold the mealworms, but large enough to allow the bran flakes to fall through. Again record weights and lengths as on the first day, but be sure to separate the weights and length of the life stages where present. Use the additional data sheet (week two) to record data for the study of the development to pupal stage and larval survival. Put team data on the board for lab. Data exchange. See additional data sheet (week two).

Place all bran, bodies, and parts along with the living members of each population back into the jar. Add new moist paper. If potato was present, then add a fresh 20 g. piece and throw away the old potato.

Place the populations back into their respective growth temperatures until the following week.

Third, Fourth and Fifth Sampling Days (Weeks three, four and five) – Repeat the weighing and measuring of the mealworms in the same manner as above. Record weights and lengths on the master data sheet and calculate the averages. Use the additional data sheet to record the numbers of larvae, pupae and adults for each of the populations. Put results of team data on the board for lab. Data exchange. See additional data sheet (Weeks, three, four and five). Each of the last three sampling days should require only about an hour of the lab. Time. This will vary with the number of students available in the laboratory.

Student Report:

The report should include appropriate tabular and graphic presentations of the data to support the discussion of the results.

Because the methods and materials as well as the purpose were stated in the assignment of this experiment, the body of the report will consist primarily of a discussion of the results and not a repeat of information already outlined in detail. The student is instructed to limit the discussion of results to two concise and precise pages. There is on the other hand no limit to the supporting graphs and tables which the student is encouraged to present. The student is encouraged to utilize the data obtained and not summarize the works of others. The limitation on pages of discussion are designed to force the student to come to grips with the numbers and mass of data rather than skirt the issue with verbage.

Results

Because the temperature differences are exaggerated, the results are striking. The mealworm populations should be purchased as large larvae about 17-19 mm. These larvae will hold at 50 0F for the entire experiment. The large larvae will pupate in low numbers in the first week in an incubator at 82 0F and will pupate in low numbers in the second week at room temperature, about 72 0F. Mortality rates are increased in the high-density and high-temperature populations. Weights and lengths need careful interpretation. Many questions may be asked and countless data combinations are possible. This experiment presents a good exercise for the freshman student, as well as others.