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NOTES – RESEARCH, TEACHING AND TECHNICAL

Male Spermatophores in Tribolium castaneum

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In the course of investigating the processes of sperm storage and sperm precedence in Tribolium castaneum (Lewis and Austad 1990, 1994; Bloch Qazi et al. 1996), we discovered that T. castaneum males transfer sperm to females in a simple spermatophore. Description of the time course of sperm movement into the female spermatheca for long-term storage is provided by Bloch Qazi et al. (1996). Here we describe female reproductive anatomy of T. castaneum in greater detail, and provide an illustration of the spermatophore.

**Methods**

Beetles were taken from a laboratory stock culture derived from the Berkeley synthetic strain. Beetles were sexed as pupae and kept individually in flour at 29°C and 70% RH in a dark incubator. Beetles were virgins and were 1- 4 wk post-eclosion. Pairs of beetles were observed in circular mating arenas until copulation occurred, after which females were removed and dissected.

**Results and Discussion**

The spermatophore consists of a membranous sperm sac approximately 0.07 µl in volume, which is attached posteriorly to a gelatinous rod. The spermatophore is deposited in the female bursa copulatrix, and fills most of the bursa as can be seen in Figure 1.

Figure 1. Spermatophore of T. castaneum male deposited within the bursa copulatrix of a female.

The spermatophore consists of a sperm-filled membranous vesicle (visible as dark shading) that is attached posteriorly to a clear, gelatinous rod. Within 10 min after mating, sperm begin to appear within the convoluted tubules of the female spermatheca. Sperm are generally not found in the common oviduct, but here have been displaced by coverslip pressure. Scale bar is 100 µm.

Shortly after the spermatophore is deposited in the bursa, the sperm sac membrane ruptures and sperm are released. Within 10 min after mating, actively swimming sperm begin to appear within the convoluted tubules of the spermatheca, and sperm numbers in the spermatheca are stable by about 1 h post-mating (Bloch Qazi et al. 1996). The structure of the female spermatheca in T. castaneum was described by Sinha (1953) and Surtees (1961). It consists of three long, blind-ended tubules connected to the anterior bursa by a short common duct about twice the width of the tubules. The spermathecal duct and tubules are surrounded by circular muscle tissue, and the entire spermatheca is enclosed within a thin muscular sheath. It is worthwhile to point out that the spermatheca and spermathecal gland were incorrectly identified by El Kifl (1953: also cited in Figures 4.4d and f in Sokoloff 1972). The function of the feather-like spermathecal gland, the duct of which opens into the female bursa through a sclerotized ring, is not known.

The spermatophore of T. castaneum is less complex than that described for Tenebrio molitor (Gadzama & Happ 1974, Happ & Happ 1975). It is not known what roles may be played in the formation of the spermatophore by the two pairs of T. castaneum male accessory glands described by Rummel and Grimnes (1991), the RAG and TAG. T. castaneum males can mate repeatedly with extremely short remating intervals, but been shown to transfer spermatophores containing decreasing sperm numbers across these consecutive matings (Bloch Qazi et al. 1996).

**Literature Cited**

Bloch Qazi, M. C., J. T. Herbeck, and S. M. Lewis. 1996. Mechanisms of sperm transfer and storage in the red flour beetle (Coleoptera: Tenebrionidae). Ann. Entomol. Soc. Amer., in press.

El-Kifl, A. H. 1953. Morphology of the adult Tribolium confusum Duv. and its differentiation from Tribolium (Stene) castaneum Herbst (Coleoptera: Tenebrionidae). Bull. Soc. Fouad 1 Entom. 37:173-249.

Gadzama, N. M. and G. M. Happ. 1974. The structure and evacuation of the spermatophore of Tenebrio molitor L. (Coleoptera: Tenebrionidae) Tissue Cell
6:95-108.

Happ, G. M. and C. M. Happ. 1975. Fine structure of the spermatheca of the mealworm beetle (Tenebrio molitor L.). Cell Tissue Res. 162:253-269.

Lewis, S. M. and S. N. Austad. 1994. Sexual selection in flour beetles: The relationship between sperm precedence and male olfactory attractiveness. Behav. Ecol.5: 219-224.

Lewis, S. M. and S. N. Austad. 1990. Sources of intraspecific variation in sperm precedence in red flour beetles. Am. Nat.135:351-359.

Rummel, R. L. and K. A. Grimnes. 1991. Preliminary comparison of the reproductive accessory glands in two species of Tribolium and their hybrids. Tribolium Info. Bull. 31: 79-82.

Sinha, R. N. 1953. The spermatheca in the flour beetle (Tribolium castaneum Herbst). Jour. N. Y. Entomol. Soc. 41:131-134.

Sokoloff, A. 1972. The Biology of Tribolium, with Special Emphasis on Genetic Aspects. Volume 1. Oxford, London.

Surtess, G. 1961. Spermathecal structure in some Coleoptera associated with stored products. Proc. Royal Entomol. Soc. Lond. Ser. A, Gen. Entomol. 36:144-152.

\*GROWTH AND DEVELOPMENT OF LASIODERMA SERRICORNE (F.)

(COLEOPTERA: ANOBIIDAE) ON VARIOUS FOOD MATERIALS

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**Abstract:** Growth and development of cigarette beetle, Lasioderma serricorne (F.) on various dried fish (Punti, Barbus ticto; Taki, Channa punctatus; Sol, C. striatus), and powdered leaves of Thankuni (Centella asiatica) and Tobacco (Nicotiana tabacum) are compared and reported in the present study. The effect of these foods is highly significant on all the growth and developmental parameters studied. Growth indices revealed that Tobacco leaf powder is the best food of Lasioderma serricorne followed by Punti fish, Thankuni leaf powder, Taki and Sol fish.

**INTRODUCTION**

Lasioderma serricorne (F.) commonly known as cigarette beetle, is one of the most destructive pests infesting a wide variety of commodities including tobacco, tobacco products, spices, timber seeds and various other stored food (Runner, 1919; Tanhet and Bare, 1951; Alam, 1971; Rizk et al., 1980; Jang et al., 1982; Malek et al.,1988). Besides these L. serricorne breeds in animal matter such as dried insects, dried fishes and fish meal, leather and wax (Howe, 1957; Lefkovitch and Currie, 1963; Ashworth, 1993). Due to its adaptation to a wide range of food stuff this beetle becomes a pervasive pest of stored commodities. However, two major constraints, i.e. low temperature and low humidity limit its propagation. Cigarette beetle is now commonly encountered and is of considerable economic importance in tropical to temperate climates (U.S.D.A., 1972). Early reports have shown that the damage is caused by the adult beetles (Jones, 1913; Howe, 1957). Recent reports have stated that adults do feed on tobacco to a limited extent (Milne, 1963; Minor, 1978), but the major damage to the stored and processed tobacco is caused by the feeding larvae (U.S.D.A., 1972; Minor, 1978; Ashworth, 1993).

Although cigarette beetle infests a large variety of food stuffs, very little information is available on dietary effects of various dried fishes, and leaves other than tobacco plant. The present investigation was therefore aimed to study the various aspects of biology of L. serricorne on three-species of indigenous dried fish, and powdered leaves of Thankuni (Centella asiatica) and Tobacco (Nicotiana tabacum).

**MATERIALS AND METHODS**

The beetles were initially collected from stored turmeric infested with L. serricorne. Stock culture was then maintained in sterilized wheat flower (95%) with brewers yeast powder (5%) at 13% moisture content. The culture was maintained in an incubator set at 28+1°C temperature and 70+2% relative humidity.

For assessing the growth and development on various food media eggs of the adult beetles were collected. A large number of beetles were put on a layer of wholemeal flower in beaker (500 ml) for oviposition. Eggs were sieved after 48 hours and incubated at 30+1°C. Five different food media were selected, viz. dried fishes (Punti, Taki and Sol), and powdered leaves of Thankuni and Tobacco. For each food medium 250 neonate larvae (24 hours old) were taken in a group of 50 in small beaker (250 ml) containing 50 gm of crushed food medium. The top of the beakers were covered with fine netted cloth secured with rubber band. Larval measurements were taken at four stages, i.e. after 7 days, 14 days, 21 days and at maturity. The larvae were weighed on an electronic balance. The length and width of the larvae were measured with an ocular micrometer (40x). Before forming a cocoon larval period was recorded, and freshly formed pupae were weighed and measured. Sex separation was conducted following Halstead (1963). Male and female pupae were placed in separate vials to record the adult emergence.

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The pupal period was recorded, and freshly formed adults (after 24 hours of emergence) were weighed and measured. The experiment was carried out in an incubator at 30+1°C, and each of the five beakers in a food medium represents a replication.

A similar set of experiment was conducted only to record the adult emergence (without any disturbance) under similar condition of temperature and humidity in an incubator. The growth indices (GI) of L. serricorne on different food media were calculated using the following formula.

 Adult recovery (%)

GI= ----------------------------------

 Total larval and pupal period

**RESULTS AND DISCUSSION**

The growth of Lasioderma serricorne larvae on three species of dried fish, viz. Punti (Barbus ticto), Taki (Channa punctatus) and Sol (C. striatus), and powdered leaves of Thankuni (Centella asiatica) and Tobacco (Nicotiana tabacum) is presented in Table 1 and those of the pupae and adults in Table 2. Analysis of variance indicated that the effect of food on the growth of the larvae in all the ages is highly significant (P<0.001). Adult and pupal measurements also varied significantly (P<0.001) due to the effect of food.

Table 1. Growth of Lasioderma serricorne larvae on various dried fish ( B. ticto, C.

punctatus and C. striatus) and powdered leaf of C. asiatica and N. tabacum

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Larval age | Food medium | Mean weight, mg ± SE | Mean length, mm ± SE | Mean width, mm ± SE |
|  | *B. ticto* | 0.42+0.025 | 0.604+0.019 | 0.229+0.013 |
|  | *C. punctatus* | 0.36+0.024 | 0.576+0.011 | 0.205+0.008 |
|  | *C. striatus* | 0. 32+0.012 | 0.420+0.009 | 0.194+0.005 |
| 7 days | *C. asiatica* | 0.38+0.020 | 0.590+0.007 | 0.213+0.010 |
|  | *N. tabacum* | 0.45+0.022 | 0.650+0.018 | 0.235+0.001 |
|  | CD at 5% | 0.068 | 0.042 | 0.025 |
|  | 1% | 0.094 | 0.058 | 0.034 |
|  | *B. ticto* | 0.84±0.089 | 1.06±0.012 | 0.445 ± 0.013 |
|  | *B. punctatus* | 0.64±0.050 | 1.03±0.007 | 0.376 ± 0.011 |
|  | *C. striatus* | 0.60±0.027 | 0.81±0.015 | 0.360 ± 0.011 |
| 14 days | *C. asiatica* | 0.70±0.031 | 1.03±0.011 | 0.388 ± 0.007 |
|  | *N. tabacum* | 0.87±0.020 | 1.11±0.010 | 0.452 ± 0.010 |
|  | CD at 5% | 0.118 | 0.038 | 0.035 |
|  | 1% | 0.162 | 0.052 | 0.048 |
|  | 1. *ticto*
 | 1.30±0.031 | 2.31±0.053 | 0.700±0.017 |
|  | *C. punctatus* | 1.14±0.050 | 2.19±0.055 | 0.673±0.012 |
|  | *C.* solaria | 1.03±0.025 | 1.88± 0.047 | 0.630±0.008 |
| 21 days | *G. asiatica* | 1.24±0.024 | 2.23±0.066 | 0.680±0.010 |
|  | *N tabacum* | 1.40±0.050 | 2.45±0.051 | 0.723±0.005 |
|  | CD at 5% | 0.090 | 0.170 | 0.032 |
|  | 1% | 0.124 | 0.234 | 0.044 |
|  | *B. ticto* | 4.10±0.171 | 3.65±0.191 | 1.2.6±0.089 |
|  Mature larva | *C. punctatus* | 3.68±0.013 | 3.31±0.117 | 1.11±0.107 |
|  | *C. striatus* | 3.46±0.172 | 2.60±0.156 | 0.92±0.062 |
|  | *C. asiatica* | 3.86±0.108 | 3.45±0.151 | 1.23±0.067 |
|  | *N. tabacum* | 4.20±0.105 | 3.80±0.174 | 1.41±0.036 |
|  | CD at 5% | 0.430 | 0.438 | 0.245 |
|  | 1% | 0.593 | 0.604 | 0.338 |

Examination of means in Table I. revealed that the weight, length and width of the 7 days to mature larvae was maximum in the larvae fed on Tobacco leaf powder followed by Punti fish, Thanlami leaf powder, Taki and Sol fish. In pupae and adults, these measurements were in the same order except the weight of male adults, where the highest value was observed in Tobacco leaf powder followed by Punti fish, Taki fish, Thankuni leaf powder and Sol fish The results on the larval measurements are in conformity with that of Sivik et al. (1957). The present findings also corroborate the results of Jones (1913) and Lefkovitch (1963) who noted significant variation in the size of the adults due to quality of larval food and female beetles were larger than the males. In the present investigation larval and pupal period showed a range of 29.10±0.74 - 36.00±1.14 days and 8.39±0.61 - 11.00±0.71 days respectively, in different foods (Fig. 1), which is very close to the findings of Samuel et al. (1984). Variation in the duration of different developmental stages depending on the quality and availability of food have also been reported by Howe (1957) and Sivik et. al. (1957)

Table. 2. Growth of L. serricorne pupae and adults on various dried fish (B. ticto, C. panda= and C. striatus), and powdered leaves of C. asiatica and N tabacuin

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Developmen tal stages | Food medium | Mean weight, rng ± SE | Mean length, nun ± SE | Mean width, mm ± SE |
|  | *B. ticto* | 3.10±0.090 | 3.12±0.130 | 1.40±0.068 |
|  | *C. punctatus* | 2.30±0.074 | 3.04±0.089 | 1.36±0.058 |
| Pupa | *C .striatus* | 2.05±0,050 | 2.61±0.096 | 1.11±0.050 |
| (Male) | *C. asiatica* | 2.78±0.161 | 3.07±0.040 | 1.37±0.055 |
|  | *N tabacuni* | 3.46±0.120 | 3.41±0.135 | 1.52±0.076 |
|  | CD at 5% | 0.3184 | 0.3083 | 0.2046 |
|  | 1% | 0.4387 | 0.4249 | 0.2819 |
|  | *B. ticto* | 4.05±0.083 | 331±0.101 | 1.52±0.078 |
|  | *C. punctatus* | 2.90±0.115 | 3.11±0117 | 1.40±0.o72 |
| Pupa | *C. striatus* | 2.60±0.106 | 2.71±0.104 | 1.21±0.052 |
| (Female) | C *asiatica* | 3.10±0.115 | 3.21±0.103 | 1.44±0.066 |
|  | *N. tabacum* | 438±0.096 | 3.50±0.143 | 1.65±0.093 |
|  | CD at 5% | 0.3327 | 0.3436 | 0.2046 |
|  | 1% | 0.4585 | 0.4735 | 0.2819 |
|  |  *B. ticto* | 2.62±0217 | 2.87±0.116 | 1.41±0.084 |
|  | *C. punctatus* | 231±0.092 | 2.80±0.110 | 1.36±0.081 |
| Adult | *C. striatus* | 2.10±0.90 | 2.20±0.101 | 1.15±0059 |
| (Male) | *C. asiatica* | 2.30±0.151 | 2.82±0.127 | 1.38±0.075 |
|  | *N tabacwn* | 2.75±0.088 | 3.00±0.171 | 1.62±0.079 |
|  | CD at 5% | 0.4220 | 0.3203 | 0.2219 |
|  | 1% | 0.5815 | 0.4414 | 0.3057 |
|  | *B. ticto* | 3.40±0.151 | 2.97±0.140 | 1.79±0.090 |
|  | *C. punctatus* | 2.88±10.139 | 2.92±0.136 | 1.51±0.062 |
| Adult | *C. striatus* | 2.55±0.097 | 2.42±0.090 | 1.52±0064 |
| (Female) | *C. asiatica* | 316±0.121 | 2.95±0.100 | 1.59±0.061 |
|  | *N tabacum* | 3.61±0.159 | 3.31±0.100 | 1.94±0.098 |
|  | CD at 5% | 0.4130 | 03703 | 0.2194 |
|  | 1% | 0.5691 | 0.5102 | 0.3024 |

Fig. 1 Larval and pupal period of L. serricorne on various dried fish ( C. punctatus, C striates and B. ticto) and powdered leaves of C. asiatica and N. tabacum

The pupal recovery and adult emergence is also affected by the food, The highest pupal recovery and adult emergence is obtained in tobacco and the lowest in Sol fish. The results of growth index have been shown in Table 3. The growth index followed the order Tobacco leaf powder> Punli fish> Thanlami leaf powder Taki fish Sol fish.

Although the highest larval and pupal period of L. serricorne are noted in Punti fish but its growth index is second in order, which proved that Punti fish is the best food medium among the dried fishes.

Table 3
Effect of various dried fish, (.8.ticto, C. punctatus and C. striatus), and powdered leaf of C.asiatica and N tabacum on the pupal recovery and adult production in L. serricorne

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Food medium | Number of Larvae | Pupation number (%) | Adult emergence number (%) | \*d-values | Growth Index(GI) |
| *B. ticto* | 250 | 140 (56.00) | 133 (53.20)  | 0.90 | 1.13 |
| *C. punctatus* | 250 | 119 (47.60) | 111 (44.40) | 2.89 | 0.98 |
| *C. striatus* | 250 | 93 (37.20) | 85 (34.00) | 5.35 | 0.80 |
| *C. asiatica* | 250 | 134 (53.60) | 127 (50.60) | 1.48 | 1.29 |
| *N tabacum* | 250 | 151 (60.40) | 143 (57.20) |  | 1.53 |

d = Standardized normal deviate.

The result varied from the findings of Howe (1957) who reported increased mortality of L. serricorne with the increase in duration of stages of life cycle due to effect of food, temperature and humidity. However, we used constant temperature in the present study, which might be a cause of variation in the results.

References

Alam, M.Z. 1971. Pests of stored grains and other stored products and their control. The Agric. Service, Agric. Dept Dhaka. pp.61.

Ashworth, J.R. 1993. The biology of Lasioderma serricorne. J Stored Prod Res. 29 (4): 291-303

Halstead, D.G.H. 1963. External sex differences in Stored Product Coleoptera. Bull. Ent. Res. 54:119-134.

Howe, R.W. 1957. A laboratory study of the cigarette beetle,L. serricorne(F.) (Col. Anobiidae) with a critical review of the literature on its biology. Bull. Ent. Res. 48: 9-56.

Jang. E. B., Lin, G.S. and Mitchell, W.C. 1982. Food preference of seven stored product insects to dried processed taro products. Proc. Hawaiian Entomol. Soc. 24: 97-107.

Jone C.R. 1913. The cigarette beetle (L.serricorne Fab.) in the Philippine islands. Philippine J. Sci. D. Gen. Biol. Ethnol Anthropol. 8:1-61.

Lefkovitch, LP. 1963. Census studies on unrestricted populations of Lasioderma serricorne (F.) (Colcoptera. Anobiidae). J. Anim. Ecol., 32: 221-223.

Lefkovitch, L.P. and Currie, J.E. 1963. The effects of food shortage upon larvae of L. serricorne(F.) (Coleoptera: Anobiidae). Bull. ent. Res. 54: 535 - 547.

Malck, M.A., Khanam, L.A.M. and Praveen, B. 1988. New record of an alternate host plant of cigarette beetle, L. serricorne Fab. (Anobiidae, Coleoptera). Bangladesh. j. zool. 16(1): 59-60.

Milne, D.L. 1963. A study of the nutrition of the cigarette beetle, Lastoderma serricorne F., (Coleoptera: Anobiidae) and a suggested new method for its controL J. Ent. Soc. S. Afr. 26: 43-63.

Minor, M.F. 1978.Do adult cigarette beetles feed? Tab. Sci. 23: 61-64.

Rizk, G.N., Mostafa, S.A.S., Shaaban, A.M. and Choniem, H.G. 1980. The population density of the cigarette beetle, Lasioderma serricorne (F.) on two types of tobacco in Egypt. Z. Ang, Ent. 90: 180-183.

Runner, G.A. 1919. The tobacco beetle: an important pest in tobacco products, U. S. Department of Agriculture, Bulletin no. 737.

Samuel, R., Prabhu, V.K.K. and Narayanan, C.S. 1984. Influence of spice essential oil on the life history of Lasioderma serricorne (F.) Entomon 9: 209-215.

Sivilk. F.P., Tenhet, I.N. and Delamar, C.D. 1957. An ecological study of the cigarette beetle in tobacco storage warehouses. J. Econ. Ent. 50: 310-316.

Tenhet, J.N. and Bare, C.O. 1951. Control of insects in stored and manufactured tobacco. U. S. Department of Agriculture, Circular no. 869.

U.S.D.A. 1972. Stored tobacco insects-biology and control. U.S.Department of Agriculture, Handbook no. 233.

Yamamoto, R.T. and Frankel, G. 1960. The suitability of tobacco for the growth of the cigarette beetle, Lasioderma serricorne. J. Econ, Ent. 53: 381-384.

Tribolium Inf. Bull. California Univ.

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\*DOSE-MORTALITY RESPONSE OF THE RED FLOUR BEETLE, TRIBOLIUM

CASTANEUM (HERBST) (COLEOPTERA: TENEBRIONIDAE) TO FENOM ®

The present investigation was carried out to find out the effect of the pyrethroid, Fenom® on various larval instars of three strains of Tribolium castaneum (Herbst), viz. CR-I, CTC-12 and FSS-II. The insecticide gave a good check of the larvae at the doses used in all the strains of the beetle.

The red flour beetle, Tribolium castaneum (Herbst) is a major pest of stored commodities, especially cereals, millets and pulses. Most strains of the beetles are resistant to chemical pesticides. T castaneum has already become resistant to several insecticides (Speirs et al., 1967; Vincent & Lindgren 1967; Champ & Campbell-Brown 1970; Dyte 1970, 1972; Cichy 1971). Champ (1986) recently reviewed the problem of pesticide resistance in grain storage pests.

When a non-specific type of resistance occurs, no permitted contact insecticides prove fully effective. Pests are normally in contact with treated surfaces for very short periods in fabric treatments. Very long exposures to higher doses are necessary to kill resistant strains (Dyte et al., 1975) and changes in the behaviour of the beetles make it even more difficult (Pinniger 1975). The use of hard-core pesticides is not permissible where safety is of prime importance. The present investigation was aimed at determining the contact action of Fenom® on the larvae of T. castaneum.

Three standard strains of T, castaneurn, viz. CR-I, CTC-12, and FSS-II, were maintained in the Crop Protection Laboratory, Institute of Biological Sciences, Rajshahi University, in 500ml beakers, each containing 80gm of flour : yeast (19:1) medium (Park and Frank 1948). Pupae were sexed (Halstead 1963) and freshly emerged adults were allowed to mate. Eggs laid by female beetles were incubated and neonate larvae were transferred to the standard flour:yeast medium to get insects of various experimental stages.

The insecticides used, Fenom® 10 EC, is a new synthetic pyrethroid produced by the CIBA-GEIGY Ltd. Its chemical component is (R,S)-α-cyano-3-phenoxybenzil (R,S) -cis, "trans" 3-(2,2- dichlorovenyl) 2,2-dimethyl cyclopropane-carboxylate. It has a mode of action typical of the pyrethroids. The doses, e.g, 2, 4, 8 and 16 ppm, were prepared by mixing the required amounts of the pyrethroid with distilled water.

Petridishes were treated by washing them with the various concentrations of the insecticide and for each stage. (Ist-6th instar larvae). Fourty insects were used for each dose and the experiments were replicated thrice. Mortality was assessed at 18-, 24-, 30- and 36-hour post-treatment and for 1st instar larvae, mortality was assessed at 6-, 12-, 18- and 24-hour post-treatment. The experiments were carried out at 30±2°C.

The LD50 values and the statistical analyses are given in Tables - 1A, 1B and IC. It is evident that a higher proportion of first-fourth instar T castaneum larvae were killed at all concentrations of Fenom®. The action was contact and age specific with a significantly lower mortality among fifth and sixth instar larvae. There was no significant variation in mortality due to strains.

Fenom® is primarily used on field pests. The only report on its use on storage pests is that of Faruki (1993). The pyrethroid gave significantly higher mortalities of the Tropical Warehouse Moth, Cadra cauttella (Walker) when used independently, and produced synergism in combination with Bacillus thuringiensis var. kurstaki and gamma irradiation. This insecticide may be used for the control of T.castaneum and other storage pests. However, further works with an array of doses are required.

The authors are thankful to the Director, Instititute of Biological Sciences, Rajshahi University, for providing laboratory facilities and to the CIBA-GEIGY (Bangladesh) Ltd. for providing the experimental sample of Fenom®.

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**References**

Champ, B.R. 1986 Occurrence of resistance to pesticides in grain storage pests. In Pesticides and humid tropical grain storage systems, In: Proc. Int. Seminar, Manila, Philippines, 27-30 May, 1985 (eds. B.R. Champ & E. Highley), pp. 229-255.

Champ, B.R. & Campbell-Brown, M.J. 1970 Insecticide resistance in Australian Tribolium castaneum (Coleoptera: Tenebrionidae). II. Malathion resistance in Eastern Australia. J. stored Prod. Res. 6 : 111-131.

Cichy, D. 1971 The role of some ecological factors in the development of pesticide resistance in Sitophilus oryzae L. and Tribolium castaneum (Herbst). Ekol. Pol. 19 : 563-616.

Dyte, C.E. 1970 Insecticide resistance in stored-product insects with special reference to Tribolium castaneum. Trop stored prod. Inf. 20: 13-18.

Dyte,C.E. 1972 Laboratory evaluation of susceptible and malathion-resistant strains of T. castaneum (Herbst) (Coleoptera Tenebrionidae), J. stored Prod. Res. 8: 103-109.

Dyte, C.E., Green, A. A. & Pinniger, D.B. 1975 some consequences of the development of insecticide resistance in stored product insects. Proc. 1st Int. Wkg. Conf stored-prod. Ent. Savannah 1974, pp. 261-271.

Faruki„ S.I. 1993. Effect of Bacillus thuringiensis var. kurstaki, synthetic pyrethroid and ionizing radiation on Cadra cautella (Walker) (Lepidoptera Phycitidae). Ph.D. Thesis, Univ. of Rajshahi, Bangladesh. 287 pp.

Halstead, D.G.H. 1963. External sex-differences in stored-products in Coleoptera. Bull Ent. Res. 54:119-134.

Park,T. & Frank, M. B. 1948. The fecundity and development of the flour beetles, T. castaneum and Tribolium confusum, at three temperatures. Ecology 29: 368-375.

Pinniger, D.B. 1975. The behaviour of insects in the presence of insecticides. The effect of fenitrothion and malathion on resistant and susceptible strains of Tribolium castaneum. Proc. 1st Int. Wkg. Conf stored-prod Ent. 1974, pp. 301-308.

Speirs, R.D., Redlinger, L.M. & Jones, R. 1971 DDT-resistant red flour beetle from a Georgia peanut seller. J. Econ. Ent. 64 1328-1329.

Vincent, L.E. & Lindgren, D.L. 1967 Susceptibility of laboratory and field collected cultures of the confused flour beetle and red flour beetle to malathion and pyrethrins. J. Econ. Ent. 60: 1763-1764.

Table 1A: LD50 values and statistical analyses on dose-mortality response of *T. castaneum* Larvae (strain ; CR-I )

|  |  |  |
| --- | --- | --- |
| Stage of Exposure time LDS° valuetreatment (hours) (ppm) | Regressionequation | X2value |
| 1st | 6 42.526 | Y=3.079+1.179x | 9.091 |
| instar | 12 9.993 | Y=3.028+1.972x | 5.685 |
| larvae | 18 2.937 | Y=4.235+1.635x | 2.919 |
|  | 24 1.379 | Y=4.738+1.874x | 12.357 |
| 2nd | 18 1.169 | Y= 4.953+0.689x | 0.114 |
| instar | 24 0.650 | Y=5.126+0.670x | 0.336 |
| larvae | 30 0.355 | Y=5.364+0.684x | 0.524 |
|  | 36 0.293 | Y=5.417+0.926x | 4.641 |
| 3rd | 18 4.301 | Y=4.350+1.026 x | 0.199 |
| instar | 24 2.986 | Y=4.497+1.060x | 1.249 |
| larvae | 30 2.209 | Y=4.603+1.154x | 1.700 |
|  | 36 1.771 | Y=4.708+1.178x | 1.727 |
| 4th | 18 11.633 | Y=3.801+1.126x | 1.183 |
| instar | 24 7.971 | Y=4.092+1.007x | 0.061 |
| larvae | 30 6.159 | Y--4.306+0.879x | 1.511 |
|  | 36 4.411 | Y=4.350+1 .008x | 1.057 |
| 5th | 18 17.116 | Y=2.689+1.874 x | 14.212 |
| instar | 24 16.977 | Y=3.067+1.571x | 9.112 |
| larvae | 30 15.032 | Y=3.386+1.371 x | 10.308 |
|  | 36 12.324 | Y=3,601+1.282x | 5.427 |
| 6th | 18 1087.849 | Y=2.457+0.838x | 1.907 |
| instar | 24 800.326 | Y=2.778+0.765x | 0.829 |
| larvae | 30 269.705 | Y=2.912+0.859x | 0.738 |
|  | 36 44.074 | Y=2.942+1.252x | 2.798 |
| Table 1B: LD50 values and statistical analyses on dose-mortality response of T. *castaneum* larvae (Strain CTC-12). |
| Stage oftreatment | Exposure time(hours) | LD50 value(ppm) | Regressionequation | X2-value |
| 1st | 6 | 64.731 | Y=3.809+0.658x | 1.015 |
| instar | 12 | 6.808 | Y=3.976+1.229x | 0.089 |
| larvae | 18 | 2.470 | Y=4.492+1.293x | 0.327 |
|  | 24 | 1.195 | Y=4.891+1.401x | 1.375 |
| 2nd | 18 | 1.497 | Y=4.864+0.774x | 0.984 |
| instar | 24 | 0.861 | Y=5.050+0.767x | 1.415 |
| larvae | 30 | 0.386 | Y=5.456+0.609x | 3.542 |
|  | 36 | 0.179 | Y=5.389+0.941 x | 7.112 |
| 3rd | 18 | 20.548 | Y=3.700+0.990x |  .958 |
| instar | 24 | 13.453 | Y=3.614+1.051x | 1.030 |
| larvae | 30 | 7.865 | Y=4.042+1.070x | 2.333 |
|  | 36 | 5.072 | Y=4.184+1.158x | 12.414 |
| *4th* | 18 | 10.953 | Y=3,341+1.596x | 10.953 |
| instar | 24 | 8.919 | Y=3.594+1.479x | 5.054 |
| larvae | 30 | 7.908 | Y=3.819+1.315x | 2.841 |
|  | 36 | 5.474 | Y=3,801+1.624x | 1.595 |
| 5th | 18 | 52.203 | Y=3.310+0.984 x | 14.882 |
| instar | 24 | 32.437 | Y=3 ,475+1.009x | 3.978 |
| larvae | 30 | 20,336 | Y=3.540+1.116x | 3.183 |
|  | 36 | 17.731 | Y=3.680+1.056x | 1.959 |
| 6th | 18 | 11308.870 | Y=2.918+0.514x | 4.421 |
| instar | 24 | 341,082 | Y=3.060+0.766x | 5.113 |
| larvae | 30 | 152.444 | Y=3.209+0.821 x | 1.949 |
|  | 36 | 62.182 | Y=3.318+0.938x | 1.857 |

Table 1C: LD50 values and statistical analyses on dose-mortality response of T. casteneurn larvae (Strain: FSS-II).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Stage oftreatment | Exposure time(hours) | LD50 value(ppm) | Regressionequation | X2-value |
| 1st | 6 | 39.791 | Y=3.1 90+1.132x | 2.947 |
| instar | 12 | 6.600 | Y=4.019+1.188\* | 0.790 |
| larvae | 18 | 0.886 | Y=5.041 +0.775x | 0.344 |
|  | 24 | 0.718 | Y=5.228+1.583 x | 3.326 |
| 2nd | 18 | 1.376 | Y=4.868+0.944x | 6.119 |
| instar | 24 | 0.523 | Y=5.251+0.892x | 4.784 |
| larvae | 30 | 0.325 | Y=5.432+0.885x | 4.010 |
|  | 36 | 0.212 | Y=5.633+0.941 x | 1.504 |
| 3rd | 18 | 5.950 | Y=4.120+1.136x | 0.486 |
| instar | 24 | 4.672 | Y=4.087+1.363x | 0.401 |
| larvae | 30 | 3.343 | Y=4.304+1.328x | 0.383 |
|  | 36 | 2.141 | Y=4.575+1.284x | 0.628 |
| 4th | 18 | 7.048 | Y=4.1 14+1.045x | 4.699 |
| instar | 24 | 5.495 | Y=4.132+1.173 x | 3.432 |
| larvae | 30 | 4.366 | Y=4.255+1.164x | 3.897 |
|  | 36 | 0.471 | Y=4.519+0.930\* | 8.236 |
| 5th | 18 | 20.794 | Y=3 302+1.203 x | 2.546 |
| instar | 24 | 20.460 | Y=3.258+1.333 x | 1.900 |
| larvae | 30 | 1.412 | Y=3.570+1.091 x | 1.363 |
|  | 36 | 1.307 | Y=3.772+0.932\* | 0.823 |
| 6th | 18 | 1297.592 | Y=2.318+0.862x | 0.378 |
| instar | 24 | 677.092 | Y=2.788+0,781\* | 3.976 |
| larvae | 30 | 100.200 | Y=2.596+1.202x | 3.522 |
|  | 36 | 51.132 | Y=3.239+1.030x | 5.837 |

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\*Histological characterization of the reproductive accessory complex of Tribolium anaphe (Coleoptera:Tenebrionidae)

Introduction

It has been a continuing task in our laboratory to examine the reproductive accessory complexes within various Tribolium species. In general, male beetles of the family Tenebrionidae possess two pairs of paired glands: long thin tubular accessory glands (TAGs) with short, uniform cells and a thicker set of glands with various types of long thin cells and opaque secretions. The second sets of glands have been named according to their general shape. Thus, these glands have been identified as BAGs, or bean-shaped accessory glands in Tenebrio molitor (Dailey, et al., 1980), RAGs, or rod-shaped accessory glands, in Tribolium anaphe, (Hafeez and Gardiner, 1964), Tribolium castaneum (Murad and Ahmad, 1977) and Tribolium freemani (Rummel and Grimnes, 1991) and PAGs, or pear-shaped accessory glands, in Triboliurn brevicornis (O'Dell, et al.,1990).

Our research has focused on both gland morphology and on characterizing the cells which compose the two glands in the complex in several species within the genus Tribolium. Previous work on beetles in the castaneum species group and the brevicgrnis species group indicate that the reproductive glands are very similar. Five distinctly different cell types were found in the PAGs of L brevicornis (O'Dell, et al., 1990; Sevener, et al.,1992) and in the RAGs of 1,, freemani (Roberts and Grimnes, 1994). To date, we had not yet fully investigated a member of the confusum group, and so we undertook a histological study of the accessory reproductive complex of T. anaphe.

Materials and Methods

All insect colonies were maintained at 30°C on a 19:1 mixture of whole wheat flour:Brewer's yeast. Adult beetles were collected from flour and dissected. For whole mount staining, glands were stained immediately in 0.03% Oil Red O (ORO) in 70% ethanol and later destained and stored in 30% ethanol. For serial sections, gland were preserved in 10% formalin, dehydrated through xylene and embedded in Paraplast. Sections (6 um) were stained in Mallory's trichrome (Gray, 1964), Cason's trichrome (Kiernan, 1990) or Periodic acid-Schiff’s reagent (using a kit obtained from EM diagnostics through Baxter),

Results and Discussion

The methods used in this study demonstrated that five distinct cell types exist in the RAG, or rod-shaped accessory gland of T. anaphe (Figure 1). Cell types were numbered in a manner consistent with the cell types of T. freemani and T. brevicornis from the references above. The results of each staining sequence will be discussed below. Diagrams are shown of the dorsal side of the RAG only, No data are reported for the TAG of T. anaphe, since in many cases the tissue did not preserve well.

Oil Red O, a lipid stain which partitions into non-polar materials within cells, was found in two distinct locations in the RAG (Figure 2). One intensely staining patch of cells (type 5) was located at the posterior end of the gland, close to the junction of the seminal vesicles and the ejaculatory duct. A second, larger group of cells across the middle of the gland was also stained (type 2). Only whole mount studies were performed with this stain as the solvents for histology often extract non-polar materials and render staining impossible.

Mallory's trichrome was differentially taken up by cells of the RAG (Figure 3). The most posterior cell type (type 3) remained relatively clear of stain. Intense orange stain was detected in a narrow band (type 4 cells) which separated cell types 2 and type 5. The other three cell types seemed to stain blue, but differed in their uptake of the stain. Type 5 cells, which ran along the medial center of the gland, stained an intense dark blue, while in the main body of the gland (type 2) the cells often contained yellow inclusions. The most anterior cells of the gland (type 1 cells) picked up a faint but distinctive blue stain. The secreted material in the lumen was brightly stained with the same colors observed in the cells, indicating that the colors are being taken up, in part, by secretory molecules destined for the female during mating.

Glands were also stained with Cason's trichrome (Figure 4). Three different cell types were stained distinctly, and their location in cross section mapped consistently with the cell type locations determined with Mallory's trichrome. The most intensely stained cell type was a dark purple color (type 4 cells), and cells at the anterior tip of the gland were a pale blue (type 1). A third patch of cells stained a strong blue (type 5). With Cason's stain, neither cell type 2 or cell type 3 picked up any stain.

The last stain used in this study was the Periodic acid-Schiffs base reaction (PAS), which stains carbohydrates and carbohydrate-containing macromolecules like glycoproteins. The results are summarized in Figure 5. Cell type 4 was stained deep purple and this is a positive stain for carbohydrate presence, possibly indicating the presence of secretory glycoproteins. Two other cell types were distinguished, type 3 (faint pink) and type 5 (grey blue), but type 1 and 2 both stained a strong magenta color and appeared identical on the basis of PAS staining. Some of the lumen contents also stained positive with PAS, indicating carbohydrate material of some kind is being passed to the female. This finding is consistent with the PAG of T. brevicornis (Sevener, et al,1992) which contains a single cell type loaded with PAS-positive material, possibly secretory glycoproteins.

Conclusions

In the course of this study, we have identified five distinctly different cell types in the rod-shaped reproductive accessory gland of T.anaphe. These findings are generally quite consistent with the characterizations of T. brevicornis, and T. freemani, in number of cell types, general location of cell types and staining phenomena seen. Although there are differences between the three species, it is clear that they are well adapted to the function of producing proteins and other secretions to pass to the female during mating. The identity of individual cell components has not been established, but all three species groups demonstrated antigenic similarity based on ELISA results (Clayton, et al., 1992). Detailed immunohistochemistry studies to further characterize the glands of Tribolium, species are currently underway.

Literature cited

Clayton, L. M., F,V. Vander Jagt, T. L. White, and K.A. Grimnes. 1992.

Relationships among proteins in male accessory reproductive gland complexes of several Tribolium species as analyzed by an enzyme-linked irnmunoabsorbant assay (ELISA). Tribolium Inform. Bull. 32:68-71.

Dailey, P.J., N. M. Gadzama, and G. M. Happ. 1980. Cytodifferentiation in the accessory glands of Tenebrio molitor. J. of Morph. 166:289-322.

Gray, P. 1964. Handbook of Microtechnique. McGraw-Hill, New York.

Grimnes, K.A. and G.M. Happ. 1986. A monoclonal antibody against a structural protein in the spermatophore of Tenebrio molitor, Insect Biochemistry. 16:635-643.

Grimnes, K.A., C.S. Bricker and G.M. Happ. 1986. Ordered flow of secretion from accessory glands to specific layers of the spermatophore of mealworm beetles: Demonstration with a monoclonal antibody. J. Exp. ZooL 240:275-286.

Hafeez, M. A. and B. G. Gardiner. 1964. The internal morphology of the adult of T. anaphe Hinton (Coleoptera:Tenebrionidae). Proc. R. ent. Soc, Lond. 39:137-145.

Kiernan, J.A. 1990. Histological and histochemical methods: Theory and Practice, 2nd ed. Pergamon Press, Oxford.

Murad, H. and I. Ahmad. 1977. Histomorphology of the male reproductive organ of the red flour beetle, Tribolium castaneurri L. (Coleoptera: Tenebrionidae). J. Anim. Morphol, Physiol. 24:35-41.

O'Dell, M., L. Paulus, and K. Grimnes. 1990. Preliminary characterization of the male accessory reproductive glands of Tribolium brevicornis (Coleoptera: Tenebrionidae). Tribolium Inform. Bull. 30:55-57..

Roberts, M. M. and K.A.Grimnes, 1994. Histological evidence for five cell types in the male accessory reproductive glands of Tribolium freemarLi (Coleoptera:Tenebrionidae) Tribolium Inform. Bull. 34: 72-74.

Rummel, R.L. and K.A. Grimnes. 1991. Preliminary comparison of the reproductive accessory glands in two species of Tribolium and their hybrids. Tribolium Inform. Bull. 31:79-82.

Sevener, J,D., N.N. Dennard, and K.A. Grimnes, 1992. Histological and histochemical evidence for an additional cell type in the male accessory reproductive glands of Tribolium brevicornis (Coleoptera: Tenebrionidae). Tribolium Inform. Bull. 32:93-95.

Figure 1. Cell type locations in the RAG of T. anaphe Dorsal view, TAGs on opposite side (not shown)

Figure 2. Oil Red 0 staining of T. anaphae RAG

Figure 3. Mailory's trichrome staining of I tophe RAG

Figure 4, Cason's trithrome staining of T. anaphe RAG

Figure 5. PAS staining of T. anaphe RAG

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Confocal imaging of Tribolium castaneum embryos

Introduction

Tribolium has recently emerged as an important organism for comparative studies of embryonic patterning (for reviews see Brown and Denell, 1996 and Denell et al., 1996). Its short life cycle, ease of culture and amenity to genetic studies have contributed to its impact in this field. To facilitate our study of Tribolium embryogenesis, we have developed a method to visualize embryos by fluorescence confocal microscopy. Confocal microscopy provides better resolution than conventional epifluorescence microscopy because whole-mount specimens are optically sectioned, and only fluorescence originating in the focal plane is seen (Cheng and Kriete, 1990). A series of optical sections is then used to form a computer-generated three-dimensional reconstruction of the embryo. The fluorescent dyes commonly used to visualize nucleic acids (e.g. Hoechst and DAN) are excited by wavelengths of light in the UV range. As UV lasers are not standard equipment on most confocal microscopes, it is desirable to use a nucleic acid-specific fluorophore with an excitation wavelength which can be produced by an Argon-Krypton laser (488, 568 or 647 nm). We have used both propidium iodide (Sigma) and YOYO-1 (Molecular Probes, Inc.) and find that YOYO-1 gives a sharper image.

Materials and Methods

Beetles were allowed to lay eggs for one hour at 31°C in Gold Medal flour supplemented with five-percent fine sifted yeast. Eggs were then collected by sieving and allowed to develop to the desired stage at 31°C. The eggs were dechorionated for two minutes in 50% bleach and washed thoroughly with water. The eggs were shaken for 20 minutes in a 1:1 mix of heptane and fixative (1X PBS; 4 % formaldehyde; 50 mM EGTA). The lower (fixative) phase was removed and one volume of methanol was added. The eggs were shaken by hand for 1 minute to devitellinate the embryos. As much of the upper phase (heptane) as possible and half of the methanol was removed. Two volumes of methanol were added and the tube was shaken to dissolve any remaining heptane. The embryos were transferred to 1.5 ml eppendorf tubes and stored at -20°C.

Embryos were rehydrated for 5 minutes in 50% methanol/50% PBS + 0.01% Triton-X-100 and then twice for 5 minutes in PBS + 0.01 % Triton-X-100 (PBT). Embryos were treated with 100 ug/ml RNase A in PBT for 30 minutes at 37°C, rinsed three times in PBT and stored at 4°C in PBT.

Embryos were washed twice for 5 minutes in PBT. PBT was removed from the tube and replaced with 100 ul 50% glycerol in PBT + 2 ul YOY0-1 (diluted 1:100 in PBT). The embryos were transferred to a Probe-Clip Imaging Chamber (RPI) and viewed on a Zeiss invert confocal laser scanning microscope (LSM-410) using the following settings: Argon laser 488 nm; Excitation filter BP 485; Dichroic Mirror FT 488/568 to objective; no dichroic mirror between objective and photomultiplier tubes (PMT's) such that all light passing through Emission Filter LP 590 is detected by PMT I. 3-D reconstructions were made from optical sections using Carl Zeiss LSM software. Images were saved as TIF files, edited for contrast in Adobe Photoshop and printed on a Tektronix IISDX dye sublimation printer.

**Results and Discussion**

Three-dimensional reconstructions of 16 and 18 hour embryos treated with YOYO-1 are shown in Figure 1. YOYO-1 is a dimeric cyanine nucleic acid stain which is excited at 488 nm and fluoresces in a range from 480 - 600 nm. As YOYO-1 binds both DNA and RNA, we have found that embryos must be treated with RNase to avoid ubiquitous fluorescence. In these embryos, distinct nuclei can be identified in the yolk, amnion, and serosa. The large size of nuclei in the amnion and serosa is probably a result of their polyploid state (Falciani et al., 1996). The shape of the embryo proper is revealed by the fluorescence of its small, densely packed nuclei. Features such as segmental divisions and the ventral midline are clearly seen.

Confocal microscopy is proving extremely valuable in studying details of embryogenesis. Three-dimensional images aid greatly in analyzing development of mutant embryos. In addition, fluorescent staining of nuclei can be paired with fluorescent or non-fluorescent in situ hybridization, allowing gene expression patterns to be viewed three-dimensionally. Although propidium iodide has been widely used to visualize nuclei in insect embryos, to our knowledge this is the first report of YOYO-1 being used for this purpose. We have used both dyes and find that YOYO-1 staining produces sharper images. This staining method should be applicable to embryos of other insects, including Drosophila.

**References**

Brown, S.J. and Denell, R.E. (1996) Segmentation and dorsoventral patterning in Tribolium. Seminars in Cell & Developmental Biology 7, 553-560.

Cheng, P.C. and Kriete, A. (1990) Image contrast in confocal light microscopy. In: "Handbook of Biological Confocal Microscopy" J.B. Pawley, ed. Plenum Press, New York.

Denell, R.E., Brown, S.J. and Beeman, R.W. (1996) Evolution of the organization and function of insect homeotic complexes. Seminars in Cell & Developmental Biology 7, 5.27-538.

Falciani, F., Hausdorf, B., Schroder, R., Akam, M., Tautz, D., Denell, R., and Brown, S. (1996) Class 3 Hox genes in insects and the origin of zen. Proc. Natl. Acad. Sci. USA 93, 8479-8484.

**Figure 1.** Three-dimensional reconstructions of 16 (A-C) and 18 (D-F) hour Tribolium embryos treated with the fluorescent nucleic acid stain YOYO-1 and visualized by confocal microscopy. In all cases, anterior is to the right. A) Lateral view of a 16 hour embryo. At this stage the germ band extends around both poles of the egg. The nuclei of the amnion and serosa can be seen overlying the embryo proper. B) Ventral view of a 16 hour embryo. The gnathal and thoracic segments have formed. C) Dorsal view of a 16 hour embryo. Only the head lobes and the tail are visible. The dense yolk does not allow the rest of the embryo to be seen. D) Lateral view of an 18 hour embryo. The germ band has extended further around the perimeter of the yolk. E) Ventral view of an 18 hour embryo free of yolk. Segmentation is apparent throughout the germ band. The first thoracic segment is indicated by an arrow. The ventral midline is also distinctly visible. Appendage buds are forming on anterior segments. F) Dorsal view of an 18 hour embryo. During this phase of germ band elongation, the caudal end of the embryo has moved on the along the dorsal surface of the egg, and is now closer to the head.

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\*Interactions in Tribolium: Competition or predator – prey?

Population biologists have developed classification systems to define rigorously social interactions between lower organisms (Haskell 1949; Burkholder 1952. Odum (1959, 1971) reported nine interactions, while Malcolm (1966) lists ten. Some of the interactions between associated populations are of benefit (+), others are harmful (-) while other interactions are neither beneficial nor harmful (0). In comensalism one species benefits, but the other is unaffected (represented by (+/0); in competition both species are harmed in some way(-,-). In predator/prey or in parasite-host interactions one species benefits and the other is harmed (+,-) (one species serves as food for the other).

Abrams (1987) has criticized the attaching of symbols to the variously named interactions because the benefit or harm of a species is vaguely defined. Nevertheless, Keddy (1989) citing Haldane (1985) aptly states: "All human concepts are only limited attempts to organize a complexity beyond the organizational capacities of our nervous system, so we should be realistic about why we need definitions and proceed with the task at hand-- to use the definition as an initial reference point for studying nature. We can expect our definitions to evolve as we learn about the phenomenon itself".

A case in point is our understanding of the type of interaction in flour beetles of the genus Tribolium. For many years the late Thomas Park, his students and collaborators studied the interspecies interaction of fribolium castaneum (CS) and T. confusum (CF), two cosmopolitan species of stored products which infest and spend most of their lives tunneling the flour in which they live. Park studied these beetles under different conditions of temperature and relative humidity. As the reviews of King and Dawson (1972) and Sokoloff (1975) have shown, from this early work he concluded that the interaction was one of competition: one of the species or the other was eliminated depending on the environmental conditions used. In the mid-sixties Park at al. (1965) and Sokoloff and Lerner (1967) independently came to the conclusion that the interaction observed when T. castaneum and T. confusum are incarcerated in the same vial is a predator-prey interaction and not (as originally assumed by Park and his collaborators and others) competition. Sokoloff and Lerner thought that under certain conditions (such as rearing T. castaneum and T. confusum in whole wheat flour enriched with brewers yeast) at 290C and 70% R.H. the interaction was one of mutual predation because food was present in abundance. Under the same conditions but utilizing other media such as corn, T. castaneum was eliminated by CF by competition rather than predator-prey interactions, since food was still abundant, but for CS certain required nutrients were in limited supply. Thus the cannibalism of eggs or other non-feeding stages by CS should greatly increase when CS and CF are reared in corn. If this is true, then competition would be at an entirely different level than usually considered, i.e. not for food as such, but for certain nutritional requirements in short supply in the food. Evidence that a shortage of these requirements causes CS to become a more active cannibal was obtained by Inouye and Lerner (1965).

At this time the information that rearing T. castaneum and T. cenfusum at 25°C. or at 30°C together produced predominantly a predator-prey interaction, but rearing the same species together at 350C produced predominantly a competition interaction was not available. This information was obtained experimentally by Bowker 1978). Unfortunately, the paper by Bowker (perhaps because of its title or the journal where it was published) did not attract the attention of Triboliumists: Her paper is not cited by any of the papers I have seen in the last 20 years. But it seems to me that her findings provided a means of determining what kind of interaction is taking place in the experiment. It will not be a black and white criterion, but it is the best we have so far: if the flour beetles are reared at 25o or 30o C. then predator-prey interactions predominate. If the beetles are reared at a temperature higher than 30°C then competition interactions predominate. The use of 35°C or higher is not recommended because rearing CS at this temperature decreases viability and increases sterility. Some people fail to see any difference in these interactions, but to put it simply, in predator-prey one species survives at the expense of the other. In competition both cohabiting species may survive, but there may be a reduction in the weight or biomass of the survivors, and as density increases mortality and other traits may be affected. If the competition is mild, both competing species survive. If the competition is severe, both may survive, one may survive or neither may survive depending on the conditions at which the competitors are placed and their physiological response to those conditions. Neither Park et al. nor Sokoloff and Lerner (1967) at that time could suggest a method of insuring which interaction was taking place. This was because, as Sokoloff and Lerner pointed out, competition and predator-prey interactions can be modified by the same factors. For T. castaneum and T. confusum these factors were:

1. Selection for a particular genotype affecting productivity, developmental rate, and competitive ability.
2. Food.
3. Initial density.
4. Other environmental factors: presence or absence of disease, temperature differences, and differences in relative humidity.

Bowker's (1979) experiments and conclusions, in my opinion, at least provide the future experimenters an a priori knowledge of the type of interaction that will occur in the experiments by choosing the proper temperature: if the experimenter wishes to study predator-prey interactions, s(he) carry out his experiments in the range of 25-30°C., where predator-prey interactions predominate. If the experimenter wishes to study competition interactions, then the temperature of choice would be 35°C., where this type of interactions predominate. The choice of temperature conditions will enable the investigator to choose a more appropriate title for the paper being submitted for publication, i.e. the inclusion of the term "competition" in the paper's title when the beetles are reared at around 35°C, and "predator-prey" when the beetles are reared at 25°C.or 30°C.

This paper is open for discussion. Anyone wishing to express an opinion about the subject is welcome to submit a paper to the Editor, for inclusion in a future issue of the Tribolium Information Bulletin.

LITERATURE CITED

NOTE: Only the references cited in this note have been included here. To insure that authors publishing their research on population interactions of Tribolium in the last 20 years are aware of the contents of this paper, the writer will send copies of this paper to the last known address.

Abrams, F.A. 1987. On classifying interactions between populations. Gecologia (Berlin) 73:272-281.

Bowker, L.S. 1979. The energetics in populations of Tribolium confusum and Tribolium castaneum. Environm. Entomol. 15, 1264-1267.

Burkholder, P.R. 1952. Cooperation and conflict among primitive organisms. Am. Sci. 40: 601-631.

Haskell , E.F. (1947. A natural classification of societies. N. Y. Acad. Sci. Trans. Series 29:186-196.

Inouve, N. and Lerner, I.M. 1965. Competition between Tribolium species (Coleoptera: Tenebrionidae) on several diets. 3. stored Prod. Res. 1:186-191.

Keddy, F.A. 1989. Competition, Routledge, Chapman and Hall, New York, N. Y.

King, C.E. and Dawson, P.S. 1972. Population biology and the Tribolium model. Evol. Biol. 5:133-227.

Park, T., Mertz, D. B., Grodzinski, W. and Prus, T. 1965. Cannibalistic predation in populations of flour beetles. Physiol. Zool. 39:289-321.

Sokoloff, A. 1975. The Biology of Tribolium with Special Emphasis of Genetic Aspects. Oxford University Press. Vol 2.

Sokoloff. A. and Lerner, I.M. 1967. Laboratory ecology and mutual predation of Tribolium species. Amer. Nat. 101:261-276.

**A simple technique to relieve Triboliurn castaneum (Coleoptera : Tenebrionidae) of Acarophenax tribolii (Acarina : Pyemotidae)**

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Acarophenax tribolii is a common parasitic mite of different stored product beetles (Lepesme, 1944). Young females of this species are found on adult beetles and their pupae, especially where the cuticle is thin, on the inter-segmental membranes and, on the adult, on the large area of soft cuticle beneath the flight wings (Evans et al., 1961). We have observed, in case of big infestation, till about hundred of adult mites on the rear dorsal extremity of the Tribolium castaneurn adult elytras and between leg articulations. After feeding, young female leaves the host and commences to feed on the eggs of the beetle until after a few days her body becomes so distended that she is incapable of walking. On the third day, the gravid female dies and from 4-14 young females emerge through the enlarged genital orifice. The single male produced by the female fecundates these young females before they leave the parent body (Evans et al., 1961). This acari, responsible of the destruction of newly laid eggs, is therefore very damaging in laboratory populations.

We have experimented a method to eliminate this acari from T. castaneum rears. Infested adults were isolated in an open Petri dish. The dishes were placed during 10 hours in a dessicator containing a 5 % formol solution. This first treatment permitted to kill all the acari present upon the beetles and then to diminish the eggs destruction. The adults were transfered in fresh rearing medium (wheat flour and brewers yeast - 10/1) for 24 hours. After these laying period, eggs were isolated and placed in the above disinfection conditions during 6 hours. Next this second treatment, eggs were incubated in a fresh rearing medium at 30 ± 3°C and 60 ± 5 % HR. A new culture could then begin.

All the material used and incubators were also disinfected by maintaining it at 60°C during 12 hours.

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References

Lepesme, P. (1944). Les coleopteres des denrees alimentaires et des produits industriels entreposes. Encyclopedie Entomologique, Serie A, XXII. Ed. Lechevalier, Paris. pp. 173-178.

Evans, G.O., Sheals, J. G. & MacFarlane, D. (1961). The terrestral acari of the British isles. Volume I : An introduction to their morphology, biology and classification. Ed. Adlard and son, Dorking. pp. 173-174.