TRIBOLIUM INFORMATION BULLETIN

Volume 38

1998

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\*Tribolium castaneum co-existence with barklice and the Sawtooth grain beetle\*

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Introduction

Species co-existence may be mediated by direct interaction or through indirect competition for resources (Brereton 1962). Tribolium castaneum may exclude other competitors by preying upon their immature stages and by rapidly exploiting food resources (Park et al. 1970). This reports on the co-existence of the (Ga-1) strain with the sawtooth grain beetle, Oryzaephilus suranemensis, (STGB) and a psocid, Liposcelis sp.

Methods

All insects were kept in continuous darkness, in an environmental chamber at 32 + 2 degree C and 70 + 5% R.H. Initial colonies were obtained from the US Grain Research Laboratory in Manhattan; K.S. Standard film vials with numerous pin holes for ventilation were used to confine the populations. Diet consisted of standard 95% wheat flour and 5% brewer’s yeast. Five vials of 8 g were inoculated with 10 adult Ga-1 and 20 adult STGB. Also, five vials of 8 g were inoculated with 10 adult Ga-1 and about 20 adult psocids. In co-existence trials diet was replaced each month using a No.80 sieve, soft forceps and a camel’s hair brush.

Results and Discussion

In the co-existence trials, Ga-1 eliminated the STGB within 7 months and psocids within 3 months. In both treatments, Ga-1 seemed to prevent reproduction of the other species (Collins 1989). Although Ga-1 was never seen preying upon live members of the other species, the destruction of inactive stages is the probable explanation for the success of Ga-1 (Park et al. 1971, Rich 1956). Therefore, the results may be viewed as predator-prey interaction (Sokoloff personal communication, 1998). Finally, the STGB adults could obtain refuge on the sides and lid of the vial which may explain their extended survival over the psocids.

 REFERENCES

Brereton, J.G. 1962. “A Laboratory study of population regulation in Tribolium confusum.” Ecology 43 (1) : 63-69.

Park, T., J.R. Ziegler, D.L. Ziegler, D.B. Mertz, 1971. “The cannibalism of eggs by Tribolium larvae.” Phys. Zool. 41 37-58.

Park, T., M. Nathanson, J.R. Ziegler, D.B. MERTZ, 1970. “Cannibalism of pupae by mixed-species populations of adult Tribolium.” Phys. Zool. 43: 166-183.

Rich, E.R. 1956. “Egg cannibalism and fecundity in Tribolium.” Ecol. 37 : 109-20.

Collins, P.J., J.C. Mulder, et al. 1989. “ Variation in life history parameters of Oryzaephilus surinamensis (L) (Coleoptera : Silvanidae).” J Stored Prod. Res. 25 (4) : 193-199.

\*COMPARED DEVELOPMENT TIME FOR THREE STRAINS OF TRIBOLIUM CASTANEUM\*

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Introduction

Development time is a critical component of population growth rate. This, in turn is critical to the understanding population interaction and dynamics (Park et al. 1970).

I measured the egg to adult development time for three strains of Tribolium castaneum (hereafter Tc): Sooty (s), Charcoal (Chr), and wild-type Georgia (Ga-1).

Methods

All insects were kept in continuous darkness, in an environmental chamber at 32 + 2 degree C and 70 + 5% R.H. Initial colonies were obtained from the US Grain Research Laboratory in Manhattan, KS and allelism tests have not been performed. Standard film vials with numerous pin holes for ventilation were used to confine the populations. Diet consisted of standard 95% wheat flour and 5% brewers yeast. In competition trials diet was replaced each month.

Development of Tc was started by placing approximately 20 adults in 8 grams of diet overnight. On the next day, the adults of unknown sex were removed and the unknown number of eggs were left to develop into adult beetles. Immature cannibalism was not controlled. This procedure was repeated 5 times using different Tc adults each time. The vials were checked daily for emerged adult beetles. ANOVA and distribution fitting was performed with Statistica.

Results and Discussion

The table below describes the results.

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Strain Total number of adults Fitted gamma distribution

 Emerged parameters scale, shape
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Ga-1 129 0.403, 64.95

Chr 156 0.073, 340.52

S 34 0.096, 247.25
\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

The distribution describes the skewed development times and can be used to compare with other researcher’s data. Note, the effects of interference and cannibalism probably play a role in the emergence time distribution (Park et al. 1971, Rich 1956). The interesting point from this experiment is that the wild derived Ga-1 strain took longer and had significantly more variation in emergence time than the other 2 strains. One of my goals with my simulation work is to understand the mechanism(s) for this variation.



 Main Effect F (2,316) = 64.94; p .0001

REFERENCES

Brereton, J.G. 1962. “a laboratory study of population regulation in Tribolium confusum.” Ecology 43 (1): 63-69.

Park, T., J. R. Ziegler, D.L. Ziegler, D.B. Mertz, 1971. “The cannibalism of eggs by Tribolium larvae.” Phys. Sool. 41 : 37-58.

Park, T., M. Nathanson, J.R. Ziegler, D.B. Mertz, 1970. “Cannibalism of pupae by mixed species populations of adult Tribolium.” Phys.Zool. 43: 166-183.

Rich, E.R. 1956. “Egg cannibalism and fecundity in Tribolium.” Ecology 37: 109-20.

Technical Note submitted for publication in TIB38

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\*TOTAL WEIGHT LOSS (TWL) AN EASY TO SCORE, FITNESS RELATED HERITABLE TRAIT IN TRIBOLIUM CASTANEUM HERBST.

The trait TWL is a measure of the metabolic activity of the offspring larval biomass produced by a couple, in a vial with culture medium, in a dark incubator at 70% relative humidity and 33 degree C. The culture medium consists of 95% whole wheat flour and 5% dried brewer’s yeast powdered. An accurate and uniform quantity of medium per vial is not needed, although it could be useful in some instances. With a simple reformed spatula we load each vial with approximately 3.0 gr. (3.0 to 3.7) of medium per vial. The trait is scored as follows:

We use a scale weighing to the nearest mg. up to 30 gr. A single vial is weighed in less than 1 second. A vial with medium and lid, weighs approximately between 14 and 20 gr. To prepare the medium the flour is heated and desiccated. The vials with the medium, but without insects, are allowed to equilibrate with the moisture in the incubator for 10 days. Due to moisture absorption the flour weight increases approximately in a 5%. Once the weight stabilizes the parent(s), either a couple or just a fecundated female, are placed in the vial. Within 48 hours after the parent(s)’s introduction, the vial weight W is scored. Twenty one days (any other precise number of days would be correct), after the parents were introduced, the weight W is scored again. The total weight loss is TWL = W - W. TWL can be scored also in vials with only (one or more) males or only virgin females (when working with a single insect a scale weighing to the nearest 0.10 mg should be used and at least 3 gr. of medium to stimulate egg laying), or in larger vials or petri dishes with more than two insects. In a trial with 101 vials, each with a single male, and 101 vials, each with a single virgin female, TWL was scored at different intervals during 60 days. The results, given in the figure, show a clear more intense metabolic activity of virgin females. Virgin-female-vials can easily be sorted out in only 15 or 20 days with reasonable accuracy. In some situations TWL might be used to sort out virgin females from males.

A single adult sampled from any population of adults is expected to be a fecundated female with probability ½. Since scoring TWL is so fast and cheap, sexing is not needed with an adequate design.

The genetic properties of TWL that follow have been estimated in the Tribolium astaneum Herbst Consejo population, captured near Madrid (Spain) and maintained in a cage in our laboratory without artificial selection since 1964.

1. Working with couples or single fecundated females, TWL in our population shows a considerable inbreeding depression and strong heterosis. The character is, to a considerable extent, influenced by the maternal genotype, and the heterosis is very large and highly significant when F1 parents are crossbred. TLS of the progeny being in many instances larger than 4 times that of the best parent.
2. The estimated realized heritability in our Consejo population, measured as response over selection differential, is h = 0.17. Response and selection differential refer to TWL, a function of the reproductive capacity of the parents, which is mainly dependent on the maternal genotype, and also a function of the viability and metabolic activity of the larvae, which depend on their own genotype.

Others properties of the character TWL can be found in : Ruano, R.G. and L. Silvela and C. Lopez-Fanjul and M.A. Toro 1996 “Changes in the additive Variance of a fitness-related trait with inbreeding in Tribolium castaneum” J. anim. Breed. Genet. 113 : 93-97.

TWL mgs. Of single males and virgin females



Technical Note submitted for publication in TIB38

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\*CONSTRUCTION OF VERY SMALL PLASTIC VIALS FROM 1, 5 ML. EPPENDORF MICROTEST TUBES, TO BE USED WHEN, WORKING WITH SINGLE INSECTS, AN ABOVE AVERAGE ACCURACY IS NEEDED.

Many times, working with Tribolium or other small insects, an above average accuracy is wanted when measuring precocity, number of stages, feed consumption, TWL etc, usually working with single insects per vial. Very small vials are needed to control errors in weight below 0.005 mg., among other things because scales with such accuracy usually do not accept weights above 3 gr. In this note we describe a way to make such vials from 1, 5 ml. microtest tubes common in most laboratories.

Handling a single insect, pupa or larva plus the medium in a complete microtest tube is not easy because of the lid that doesn’t let the air in and the conic shape of the bottom. If half of the test tube is cut off, the part with the lid could be used as a small vial, with the closed lid becoming the bottom of the vial (Fig. 1, 1-2).

As a safety precaution, to prevent unwanted injuries, the test tube could be handled with a rubber tube holding it, or with a stick of any material (a common pen adjusted with adhesive tape to the diameter of the tube) introduced into the tube. (Fig. 1, 3-4). Wooden devices with drills are easier to use (Fig. 1, 5-6-7).

Any cutting instrument with a very sharpened blade could be used.

The resulting vials without lids could be stored in large transparent plastic cages to prevent contaminations. They can be handled easily with common forceps or with the hands. They can be cleaned in water and detergent in large jars, strongly shaken and then rinsed in the same jar.

The vials could be weighed with or without medium, depending on the experiment. The amount of medium per vial should weight at least 20 mg. A single egg, or better a 48h. old larva per vial can be used.

When eggs are needed they should be harvested within 24 to 48 hours, sifting as usual. The eggs are placed in common crystal vials. The eggs are usually covered with enough rests of medium for the larva to survive 48 hours after hatching. The larvae are then placed in a piece of paper and the rests of medium are cleaned off inclining and shaking gently the paper. The larvae are easily seen as they move.



Larvae like eggs are handled with small brushes. Introducing them into the small vials is facilitated using the handmade devices shown in the figure. We made them from old film cartridges (Fig. 1, 8), or other materials easy to cut like black films, or tin plate from used empty beer cans. A simplified diagram (Fig. 1, 9-10) shows how we cut the devices, cutting through the continuous lines and folding through the discontinuous lines.

These devices could also be used to fill the small vials with medium but we prefer to make small tin plate spatula. (Fig. 1, 11-12).

When scoring TWL (TIB this issue) in small vials we suggest to read the first weight within 48 h. and the second after 12 (any other precise number of days, up to 14, would be correct).

Working with large larvae or pupae a flexible soft forceps is recommended. : Ruano, R.G. 1977 “A simple technique to inmovilise Tribolium adults” TIB 20 : 151-152.

I am very grateful to my daughter Mercedes for her help with the figure.

 \*ASSESSMENT OF TRIBOLIUM CASTANEUM (HERBST) EGG WEIGHT

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Summary

We examine in this paper the relationship between the weight of egg coated with flour dust to the weight of egg cleared of flour dusts in Tribolium castaneum. The aim of this study was to know whether a bias would be induced if we correlated some of the life history traits with the weight of egg coated with flour dusts.

We found that there is no need to wash eggs before weighing. There is a significant linear relation between the two types of eggs (r2Asm = 0.7416; p = 0.000; n = 100) and (r2PRm = 0.5721; p = 0.000; n = 100). A further observation is that there is a high variability in egg weight.

Egg viability was also assessed after the washing process and compared to unwashed egg viability. The washing process leads to a high level of mortality before adult stage (10.84% mortality in unwashed eggs as compared to 57.00% in washed eggs).

Introduction

Tribolium castaneum is a worldwide pest affecting a large variety of cereal products. The economic entomologists throughout the world spend a great deal of energy in attempts to determine the presence of beetles in stored products, check their infestations, and design better and safer methods to bring them under control (Sokoloff 1972). Nowadays, in insect pest management ecological studies and evolutionary biology are combined to control insect pests. Workers use tools such as life history traits evolution in such studies. Estimation of life history traits often takes into account the relationship between egg size, egg weight to survival of larvae, development time, lifetime fecundity and/or adult longevity. In Tribolium castaneum, eggs laid are covered with a mucilaginous substance that favours fine flour dusts “coating” on them. The following experiments were carried out to determine whether the flour dusts which coat the eggs is a source of bias in establishing allometry relationship between egg and any other life cycle feature.

Material and Methods

Insect rearing

Two flour beetle strains were used in this study. One strain, PRm, was collected from

A grain store in the Philippines. The second strain, Asm, was collected from storage facilities in the Ivory Coast (Haubruge et al. 1997).

Both strains were cultured in a dark incubator, at 30 + 3 degree C and 60 + 5% relative humidity with a mixture of whole-wheat flour and brewer yeast (10/1 –wt/wt) as rearing medium.

Egg weighing (unwashed eggs (UWE) versus washed eggs (WE))

Adults of both strains laid egg in 150 mm petri dishes containing 100 g rearing medium and the eggs were collected after 24 hours by sieving them with 0.25 mm mesh sieve. Each egg was weighed, and then soaked for less than a minute in distillate water and allowed to dry on a “Teflon” covered plate and then weighed again. Eggs were weighed on a micro-scale (Sartorius supermicro) to the nearest 10 mg. One hundred eggs were weighed for each strain. We seized the opportunity to “sample” the variability of egg size within and between the strains, by calculating of the variability coefficients.

Effect of the washing process on egg viability

Fifty adult insects were placed for eggs laying in 150 mm petri dishes containing 100 g of rearing medium. After 24 hours, eggs were collected, soaked and dried as described above. Two hundred eggs of each strain were placed separately in a tube containing 0.5g of rearing medium. Eggs were kept in the same conditions as culture of Tribolium castaneum. Unwashed eggs were used as control. An egg was considered to be viable when a larva emerged. After 4 days, the number of larvae was then observed.

Results and discussion

 

Figure 1. Weight relation between unwashed eggs and washed eggs of Tribolium castaneum. (a) Asm’ correlation curve (r2 =0.7416; p=0.000; n=100); (b) PRm’ correlation curve (r2= 0.5721; p=0.000;n=100)

In both strains, there was a positive linear relationship between the UWE and WE, r r2Asm = 0.7416; r2PRm = 0.5721 respectively (p = 0.000). It was concluded that the flour dusts which coat the eggs is not a source of bias in establishing allometry relationship between egg and any other life history feature.

The results showed that egg washing process lead to high mortality (57% in washed eggs as compared to 10.84% mortality in unwashed eggs), which was certainly due to the washing process.

Handling of eggs, such as during pooling with a brush, could have contributed to increased mortality. Another probable source of mortality can be attributed to the removal of the mucilaginous substance that, probably, plays an important role in protecting eggs again environmental stress. Park (1942) used soap solution containing mercuric chloride (1:1,000) to wash beetle eggs of parasites; he found that viability was not significantly affected by this process. However, our results are not comparable to those of Park, since we did not use the same solution in the washing process.

Net weight of eggs was compared between the two strains of Tribolium castaneum. Data analysis was performed with Minitab software 10.1, using Anova oneway.

Table 1: Egg weight (in mg) of both PRm and Asm

 N = 100 PRm Asm

 Means + SE (mg) 0.0352 + 0.0007 0.0351 + 0.0009

 Coeficient of variation 20.594 27.347
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No difference was found between Asm and PRm eggs weight at level of 95% confidence, p value was higher than 0.05 (p = 0.951). We also observed that egg weight was variable in both strains. Calculation of variability coefficients reveals that egg size in Asm strain varies to a greater extent than in PRm strain (table 1).

Conclusion

Results from this tudy indicate that there is a positive linear relationship between the UWE and WE of Tribolium castaneum. Therefore, there is no need to wash eggs before performing life history experiments that take into account allometry relationship between the egg weight and the other traits.

Acknowledgements

We are grateful to FNRS for providing a grant for the micro-scale (Satorius supermicro)

This srsearch was supported by Ph. D. grants from the FRIA to L. Arnaud and by the Scientific Research Ministry of Ivory Coast to L. –K. Assie

The authors wish to thank Mrs. Jennifer Moreman for reading the manuscript.

Cited References.

Haubruge, E., L., Arnaud, & Mignon, J. (1997). The impact of sperm precedene in malathion resistance transmission in populations of the red flour Tribolium castaneum (Herbst)(Coleopters:Tenebrionidae). Journal of stored Products research 33 (2):143-146.

Park, T. (1942). Experimental studies of interspecies competition: Competition between populations of the flour beetles, Tribolium confusum (Duval) and Tribolium castaneum (Herbst). Ecological Monograph 18 (2):266-307.

Sokoloff, A. (1972). The biology of Tribolium, with special emphasis on genetic aspect. Oxford University Press.

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\*Symptoms of Triflumuron Intoxication in Larvae of Tribolium castaneum (Herbst) (Coleoptera : Tenebrionidae)

The benzoylphenyl urea (BPU) compounds inhibit chitin synthesis in insects and hence, disrupt cuticle formation mainly in the larval instars. These compounds are not lethal at short exposure, but prevent metamorphosis. Death occurs at a longer exposure mainly due to the disruptive cuticle which unable to prevent the effects of internal body pressure (Mulder and Gijswijt, 1973) during ecdysis. Larvae exposed to BPU treatment do not die until the next moult or one of the next moults (Hammann and Sirrenberg, (1980), in the mean time the affected larvae show symptoms of intoxication. BPU treatments either through diet or injection, had been reported to produce various symptoms in the larvae of different insects (van Daalen et al., 1972; Salama et al., 1976; Mauchamp and Perrineau, 1987; Clarke and Jewess, 1990; Degheele et al., 1993). Such information from stored products insects are scanty. The present work was undertaken to diagnose the symptoms of intoxication of Triflumuron (a BPU compound) in the larvae of both malathion-susceptible (FSS II) and multi-resistant (CTC 12) strains of Tribolium castaneum Herbst, a major pest of the stored food commodities.

The larvae (1st to 6th instars) of both strains of the beetle were obtained from a larval culture (Mondal, 1984) and were allowed to feed on triflumuron treated food (800 to 24,000 ppm) for 48 hours. The 1st and 2nd instar larvae of FSS II strain died at doses below 800 ppm. All the larvae dead or live were examined under a microscope to observe the effects of intoxication. The symptoms were visible in the matured larvae by eye observation.

The symptoms of intoxication were distinct in 3rd to 6th instar larvae feeding on treated diet (8,00 to 24,000 ppm) for 48 hours. The following morphological and behavioural disabilities of the treated larvae were observed in both strains:

1. The dead larvae irrespective of age/instar and dose became black and shrunken, it seemed that death had been occurred due to desiccation. More than 70% of the dead larvae were found to be attached to the old cuticle.
2. Live larvae became lethargic and ceased moving or feeding (moribund).
3. At higher doses (16,000 to 24,000 ppm), larval integument became dull red coloured with small melanized patches on abdominal terga.
4. Ecdysial line of the prothorax splitted, during moulting, but the larvae failed to moult and subsequently died 4 to 6 days after treatment.
5. Abdomen of 5th and 6th instar larvae became swollen as balloon like and when they were punctured a droplet of fluid oozed out.
6. Live 6th instar larvae fed on dosages higher than 2,000 ppm for 48 hours were transferred to untreated food after which mostly failed to pupate and died in that condition approximately within 10 to 12 days post-treatment. A few of these larvae succeeded to pupate but could not survive.

As cuticle protects the larvae from desiccation, the disruptive cuticle could not prevent the larvae from drying up, and the dead larvae became shrunken and C-shaped. Similar symptoms were reported by Mian and Mulla (1982) from stored products insects fed on triflumuron and diflubenzuron treated diet. Incomplete moulting, untimely death of larvae, several hours after removal from the treated food, torpid larvae ceased to move and feed, were also observed by Carter (1975), Salama et al. (1976), van Eck (1979) and Degheele et al. (1993) in different insects fed on BPU treated diet. Ascher et al. (1979) explained these effects as a deterioration of the mechanical properties of the cuticle. Dull coloured abdominal terga was observed in Colarado potato beetle fed on cyromazine (Sirota et al., 1993). It was suggested that the swollen abdomen of the infected larvae resulted from the increased internal body pressure of the larvae (Mulder and Gijswijt, 1973). This hypothesis was supported by the fact that when the larvae were punctured, a droplet of fluid oozed out (Cooper et al., 1983).

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The same result was observed in the present study. All the symptoms diagnosed in the larvae fed on triflumuron treated diet, were same in both strains of T. castaneum, and focused on certainty of cuticular disruption.

Acknowledgment: I would like to express my sincere thanks to Dr. B.J. Selman, Department of Agricultural and Environmental Science, University of Newcastle, Newcastle upon Tyne, NE1 7RU, UK and Professor K.A.M. Shahadat Hossain Mondal, Director, Institute of Biological Sciences, Rajshahi University, Rajshahi 6205, Bangladesh for patient reading and valuable comments on the manuscript.

References

Ascher, K.R.S., Nemny, N.E., Eliyahu, M. and Ishaaya, I. (1979). The effect of BAY SIR 8514 on Spodoptera littoralis (Boisduval) eggs and larvae. Phytoparasitica 7 (3): 177-184.

Carter, S.W. (1975) – Laboratory evaluation of three novel insecticides inhibiting Cuticle formation against some susceptible and resistant stored products beetles. J. Stored Prod. Res., 11: 187-193.

Clarke, B.S. and Jewess, P.J. (1990) – The uptake, excretion and metabolism of the acylurea insecticide, flufenoxuron in spodoptera littoralis larvae, by feeding and topical application. Pestic. Sci., 28 : 357-365.

Cooper, R.M., Lindquist, R. and Simonet, D.E. (1983) – Timing of applications of SIR 8514 for control of the Colorado potato beetle (Coleoptera : Chrysomelidae) on potatoes. J. Econ. Entomol., 76 : 565-566.

Degheele, d., Yi, S.X. and Bai, C. (1993) – Toxicity of benzoylphenylureas to the African armyworm Spodoptera exempta (Walker). Crop Prot., 12: 35-38.

Hammann, I. and Sirrenberg, W. (1980) – Laboratory evaluation of SIR 8514, a new Chitin synthesis inhibitor of the benzoylated urea class. Pflazenschutz Nachrichten Bayer 33 (1) : 1.34.

Mauchamp. B, and Perrineau, O. (1987) Chitin biosynthesis after treatment with Benzoylphenyl ureas. In: Chitin and Benzoylphenyl ureas (J.E. Wright and A. Retnakaran eds.), Dr. w. Junk Publ., The Netherlands. pp. 101-109.

Mian, L.S. and Mulla, M.S. (1982) Biological activity of IGRs against four stored Product coleopterans. J. Econ. Entomol., 75: 80-85.

Mondal, K.A.M.S.H. (1984) – A method of determining the larval instars of Tribolium castaneum Herbst (Coleoptera : Tenebrionidae). Lab. Practice., 33: 120-121.

Mulder, R. and Gijswijt, M.J. (1973). The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. Pestic. Sci., 4 : 737-745.

Salama, H.S., Motagally, Z.A. and Skatulla, S.G. (1976). On the mode of action of dimilin as a moulting inhibitor in some lepidopterous insects. Z. angew. Entomol., 80 : 396-497.

Sirota, J.M., Grafius, E., Ferrari, B., Kolarik, P., Scriber, B., Simstead, S. and Boylean-Pett, W. (1993) Control of Colorado potato beetle with Trigard. Insectic. Acaricide Tests 18 : 155-156.

Van Daalen, J.J., Meltzer, J., Mulder, R. and Wellinga, K. (1972) – a new insecticide with a novel mode of action. Nature 59 : 312 – 313.

Van Eck, W.H. (1979) – Mode of action of two benzoylphenyl ureas as inhibitors of Chitin synthesis in insects. Insect Biochem., 9 : 295-300.

Tribolium Inf. Bull. California Univ.

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\*TOXICITY OF THEVETIA PERUVIANA SEED EXTRACTS ON TRIBOLIUM CASTANEUM (HERBST) ADULTS

Natural plant protection methods have assumed a new dimension in the era when a host of synthetic pesticides is available which seems to offer an easy answer to the problems of pest management and disease control. Unfortunately these have neither solved the purely agricultural problems of the small farmers, nor they have improved their economic conditions. On the contrary, they have incurred a plethora of problems which are self-defeating.

Synthetic pesticides proved to be unduly toxic to all forms of life, ecologically disastrous, or induced insect resistance, which led to a frantic search for safer pest control agents. Botanical pesticides because of their environment-friendliness have received much recent attention. Several insecticidal agents have been isolated, identified and screened from members of many plant families (Jacobson et al., 1975; Bernays and Chapman, 1977; Doskotch et al., 1977; Jacobson et al., 1977; Shudhakar et al., 1978; Carpentier et al., 1979; Warthen, 1979; Jurd and Manners, 1980; Menn, 1980; Saxena, 1983; Arnason et al., 1989; Stoll, 1992).

The plant, Thevetia peruviana (Pers.) Schum. (Family apocynaceae), locally known as Karabi, is a large evergreen shrub and Is a native to tropical America and the West Indies, but is naturalized and cultivated in gardens and roadside plains although Bangladesh. The red flour beetle, Tribolium castaneum (Herbst) is a major stored products pest throughout the tropical and subtropical countries of the world. The present work reports the effects of the extractives of the seeds of T.peruviana on T.castaneum adults.

The three standard strains of the beetle (T. castaneum), e.g. CR-1, fss-ii and CTC-12 were reared on a whole wheat flour –powdered dry yeast (19 : 1) mixture at 30 + 2 degree C in the Insect Research Laboratory, Department of Zoology, Rajshahi University. Bangladesh. Freshly formed adults were used in the experiment. Sun-dried T. peruviana seeds were made into a fine dust. The active ingredients of the seeds were extracted with petroleum spirit (Pt. spt.), ethyl acetate (EtOAc), acetone and methanol (McOH), and were preserved at 4 degree C for future use.

The extracts were dissolved in the solvents to prepare experimental doses: 116.76, 77.84, 51.89 and 34.59 ug/ul for pt. spt. extract; 14.28, 9.52, 6.35 and 4.23 ug/ul for EtOAc extract; 15.63, 10.43, 6.95 and 4.63 ug/ul for acetone extract; and 54.49, 36.33, 24.23 and 16.15 ug/ul for MeOH extract, which were applied topically to the adult beetles to assess their mortality at 24 hr post-exposure. For each dose and strain 10 beetles were employed and the experiment was repeated three times. Groups of control insects were treated with the solvents only.

The mortality data were corrected by Abbott’s (1925) formula and were subjected to probit analyses (Finney, 1947 and Busvine, 1971).

The toxicity of T. peruviana seed extracts on T. castaneum adults is presented in Table 1. Results show that the EtOAc extracts were the most toxic to T. castaneum adults which was followed by pt. spt., acetone and MeOH. The contact toxicity of the seed extracts to various T. castaneum strains could not be generalized. It was in the order CTC-12 CR-1 FSS-II for pt. spt.; FSS-II CR-1 CTC-12 for EtOAc; CR-1 and FSS-II CTC-12 for acetone; and CTC-12 FSS-II CR-1 for MeOH respectively.

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Extractions were done with four solvents for separating different compounds from the seeds of T. peruviana; pt. spt. extracts oils, fats and fatty acids; EtOAc separates terpenes, alkaloids, flavones and steroids; acetone separates chlorophylls, dye and other alkaloids which are not soluble in EtOAc; and MeOH extacts all the remaining alkaloids and acidic compounds.

Islam (1996) evaluated the contact toxicity of the lead, seed, fruit-pericarp, stem-bark and root of the Pithraj plant, Amoora rohituka W. \* A. in the same solvents and with the same strains of T. castaneum used in the present work. The extractives proved very much promising in controlling the pest by producing high mortalities due to contact toxicity.

T. peruviana have profound contact toxicity for T. castaneum, which may also be utilized for other storage pests too.

Acknowledgements:

The authors acknowledge with gratefulness the laboratory facilities extended by the Chairman, Department of Zoology, Rajshahi University, Bangladesh.

References

Abbott, W.S. 1925. A method of computing the effectiveness of insecticides.

 J. econ. Ent. 18: 265-267.

Arnason, J.T., Philogene, B.J. R. and Morand, P. (Eds.) 1989. Insecticides of plant

 Origin. ACS Symposium Series 387. American Chemical Society,

 Washington, D.C. 213 pp.

Bernays, E.A. and Chapman, R.A. 1977. Deterrent chemicals: a basis of oligophagy in

 Locusta migratoria L. Ecol. Ent. 2 : 1-18.

Busvine, J.R. 1971. A critical review of the techniques for testing insecticides.

 CAB, London. 345 pp.

Carpentier, T.L., Neel, W.W. and Hedin, P.a. 1979. A review of host plant resistance of

 Pecan, Carya illinoensis, to insect and acarina. Bull. Ent. Soc. Am. 25:

 251-257.

Doscotch, R.W., Odell, T.M. and Godwin, P.A. 1977. Feeding response of Gypsy moth

 Larvae Lymantria dispar, to extracts of plant leaves.Ent. 6: 565-566.

Finney, D.J., 1947. Probit analysis. Cambridge University Press, London. 333pp.

Islam, M.N. 1996. Studies on the effect of extractives of different parts of

 Amoora sp. On the red flour beetle, Tribolium castaneum (Hbst.).

 Ph.D. Thesis, Institute of Biological Sciences, Rajshahi University,

 Bangladesh. 149 pp.

Jacobson, M. 1977. Isolation and identification of toxic agents from plants.

 In: Host plant resistance to pests. ACS Symp. Ser. 62: 153-164.

Jacobson, M., Redeern, R.E., and Mills, G.D., Jr. 1975. Naturally occurring insect

 Growth regulators-II. Screening of insect and plant extracts as insect

 Juvenile hormone mimics. Lloydia 33: 455-472.

Jurd, L., and Manners, G.D. 1980. Wood extractives as models for the development of new types of pest control agents. J. Agric. Fd. Chem. 28 : 183-188.

Menn, J.J., 1980. Contemporary frontiers in chemical pesticide research.

 J. Agric. Fd. Chem. 28 : 2-8.

Saxena, R.C. 1983. Naturally occurring pesticides and their potential. In: Chemistry and World food supplies: The New Frontiers. CHEMRAWN II, Manila,

 Philippines, 1982. Pergamon Press, Oxford and New York, pp. 143-161.

Shudhakar, T.R., Pandey, N.D. and Tewari, G.C. 1978. Antifeeding property of some

 Indigenous plants against mustard sawfly, Athalia proxima

 (Hymeoptera: Tenthredinidae). Indian J. Agric. Sci. 48 : 16-18.

Stoll, G. 1992. Natural Crop Protection in the Tropics. AGRECOL, Weikersheim,

 FR Germany.

Warthen, J.D., Jr., 1979. Azadirachta indica: A source of insect feeding inhibitors

 And growth regulators. U.S. Dep. Agric. Rev. Man. ARM-NE-4.

Table 1 : Toxicity of T. peruviana seed extracts on T. castaneum adults.

Solvents used Strains LD values (ug/insect) Regression equation

 CR-1 5.58 Y = 3.14 + 1.06x

Pt.spt. FSS-II 10.05 Y = 3.58 + 0.71x

 CTC-12 5.15 Y = 3.47 + 0.90x

 CR-1 0.43 Y = 4.28 + 1.14x

EtOAc FSS-II 0.36 Y = 4.59 + 0.74x

 CTC-12 0.59 Y = 4.50 + 0.64x

 CR-1 22.19 Y = 3.19 + 0.77x

Acetone FSS-II 22.19 Y = 4.19 + 0.77x

 CTC-12 14.16 Y = 2.39 + 0.03x

 CR-1 352.65 Y = 3.31 + 0.66x

MeOH FSS-II 89.88 Y = 3.51 + 0.76x

 CTS-12 87.19 Y = 3.41 + 0.82x

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Note: This paper, published in 1996 in the Tribolium Information Bulletin 36:83-85 has been shortened and somewhat modified to fit a two-page requirement for the TIB On-Line.

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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. Some interactions between associated populations are of benefit (+), other interactions are harmful (-), and others are neither beneficial nor harmful (0). In commensalism of two species one benefits while the other is not harmed (+/0). In completion both species are harmed in some way (-/-). In predator-prey or parasite-host inter-actions one species benefits and the other is harmed (+/-) (one species serves as food for the other). We are concerned here only in the last two interactions.

As the reviews of King and Dawson (1972) and Sokoloff (1975) have summarized, the late Thomas Park and his students and collaborators studied interactions between Tribolium castaneum (CS) and T. confusum (CF). He concluded that the interaction between these stored-product pests was competition; one of the species or the other was eliminated depending on the environmental conditions used. In the mid-sixties Park et al (1965) and Sokoloff and Lerner (1967) independently came to the conclusion that the interaction observed when these two species are placed in the same vial is a predator-prey interaction and not (as originally assumed by Park and his collaborators and others) a competition interaction. Sokoloff and Lerner thought that under certain conditions (such as rearing CS and CF in whole wheat flour enriched with brewer’s yeast at 29 degree C and 70% R.H. the interaction is one of mutual predation because food is present in abundance and regularly renewed, and under these conditions CS is the winner. Under the same conditions, but utilizing other media such as corn, CS was eliminated by CF. Again the amount of food is probably in excess, since once CS is eliminated CF experiences a threefold increase in population size. But here the possibility that competition has occurred cannot be ruled out, because certain nutritional required nutrients are in limited supply in corn. Evidence that a shortage of these requirements causes CS to become a more active cannibal was obtained by Inouye and Lerner (1965).

At the time when these experiments were carried out neither Park et al (1965) nor Sokoloff and Lerner (1967) had any experimental basis to show that temperature may be a useful guide to resolve what kind of interaction (competition or predator-prey) is prevailing in the experiment. Bowker (1978) showed in her measurements of energetics of populations of single and mixed species of CF and CS that when beetles are reared at 25 degree or 30 degree C predator-prey interactions predominate, while at 35 degree C competition interactions predominate. Unfortunately, her paper did not attract the attention of Triboliumists: Her paper is not cited by any of the papers on competition or other interaction studies in the last 20 years.

In my opinion, recent students of interactions in Tribolium species, judging from the contents and their titles and the temperatures at which the experiments have been carried out, have misidentified the type of interaction they are observing, perhaps because of an inadequate search of the available literature. To a certain extent reliance on the literature published and available in data bases leads to errors in interpretation such as those I have described here. I have made available the facilities of the Tribolium Information Bulletin as an open forum to discuss the topic. So far only one Triboliumist has shown interest, but the open forum will remain open for a couple of years.

Literature Cited

Bowker, L.S. 1979. The energetic in populations of Tribolium confusum and

 Tribolium castaneum. Environm. Entomol. 15 : 1264-1267.

Inouye, N. and Lerner, I.M. 1965. Competition between Tribolium species

 (Coleoptera : Tenebrionidae) on several diets.

 J. stored Prod. Res. 1 : 186-191.

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. 38 : 289-321.

Sokoloff, A. 1975. The Biology of Tribolium with Special Emphasis on Genetic Aspects.

 Oxford Univ. Press. Vol.2.

Sokoloff, A. and Lerner, I.M. 1967. Laboratory ecology and mutual predation of

 Tribolium species. Amer. Nat. 101: 261-276.

 Throne, James E. USDA-ARS Marketing and Production Research Center,

 Manhattan, Kansas

RESEARCH HIGHLIGHTS AND TECHNOLOGY TRANSFER FOR FY97:

INSECTICIDE EFFICACY. Determined that confused flour beetles are more susceptible than red flour beetles to cyfluthrin wettable powder applied on porous concrete. Red flour beetle mortality increased as the application rate and exposure interval increased. (Franklin H. Arthur).

SHOWED THAT MORTALITY of the 5th instar indianmeal moths exposed to cyfluthrin wettable powder on concrete increased as the application rate increased, but that there was no difference in either pupation or adult emergence when larvae were exposed for short intervals of 0.5 to 4 hours. After 3-6 weeks, adult emergence exceeded 20% at all application rates. (Franklin H. Arthur).

AERATION OF GRAIN could be used to manage farm-stored corn in the southern United States, but the threshold temperatures used for activating the aeration fans would have to be higher in those areas where there is an extended warm fall season. Increasing the fan speed may also be necessary to cool the corn quicker. (Franklin H. Arthur).

ASSISTED GUSTAFSON, INC., on the residual activity of cyfluthrin applied to concrete. (Franklin H. Arthur).

COLLABORATED WITH INDUSTRIAL FUMIGANTS AND WHITMIRE, INC., to evaluate a new encapsulated formulation of cyfluthrin as a wheat protectant. (Franklin H. Arthur).

COLLABORATED WITH RHONE-POULANC TO EVALUATE AN INSECTICIDE that is a member of a new class of chemicals with a novel mode of action. (Franklin H. Arthur/Richard W. Beeman).

COLLABORATED WITH MYCOTECH CORP. TO EVALUATE A STRAIN OF ENTOMOPATHIC FUNGI for use on raw grains and flooring surfaces. (Franklin H. Arthur).

COLLABORATED WITH KANSAS STATE UNIVERSITY, DEPT. OF GRAIN SCIENCE, ON AERATION STRATEGIES for farm-stored corn. (Franklin H. Arthur).

ASSISTED THE DEPARTMENT OF GRAIN SCIENCE AT KANSAS STATE UNIVERSITY in tests of the efficacy of a dichlorvos aerosol application and a heat treatment in the flour mill. (Franklin H. Arthur/Alan K. Dowdy/Michael A. Mullen)

DEMONSTRATED THE STABILITY OF MALATHION RESISTANCE in populations of

Anisopteromalus calandrae, a beneficial parasite of stored-grain pests. (James E. Baker).

COLLABORATED WITH THERMO TRILOGY CORP., Columbia, MD, about testing

Neem products for storage pest control. (James E. Baker).

DEVELOPED A LABORATORY SELECTION METHOD for increasing the frequency of insecticide resistance in the beneficial parasitic wasp, Bracon hebetor, (James E. Baker)

CLONED AND SEQUENCED ESTERASE cDNAS from malathion resistant and susceptible strains of a beneficial parasitic wasp, Anisopteromalus calandrae. DNA markers that differentiate genetic differences between the resistant and susceptible strains have been developed. (James E. Baker/Alan K. Dowdy).

GENOME MAP FOR STORED-PRODUCT INSECT PEST. Constructed a complete genome molecular map of the red flour beetle using randomly amplified polymorphic (RAPD) DNA markers, visible mutant markers, and specific gene markers. The map is now being used to facilitate map-base cloning of important insect control genes. (Richard W. Beeman).

GENE VECTORS FOR GENETIC ENGINEERING OF INSECTS. Developed gene vectors for transferring insects with novel genes. Recent improvements in transformant selection technology offer hope that potentially-useful transposon vectors will be used to genetically manipulate stored-grain insect genomes. (Richard W. Beeman).

MECHANISMS OF HYBRID INVIABILITY ELUCIDATED. Demonstrated that killer genes known as “Medea” (M) factors are required for larval mortality associated with a hybrid inviability gene (H). Knowledge of the detailed mechanisms of larval kill in the M and H systems could be exploited in new, naturally-based insect control strategies. (Richard W. Beeman).

DEVELOPED A LABORATORY TECHNIQUE BASED ON THE POLYMERASE CHAIN REACTION (PCR) for cloning insertions of foreign DNA into insect chromosomes and provided advice and materials to several university and government research laboratories that now use this technique. (Richard W. Beeman).

SCREENING OF THE INDIANMEAL MOTH FOR GENETIC FINGERPRINTS linked to resistance to the bacterial insecticide Bacillus thuringiensis was conducted. The messenger RNA that codes for aminopeptidase in Indianmeal moth was characterized. Screening of Anisopteromalus calandrae, a parasitoid of the rice weevil, for genetic fingerprints linked to resistance to Malathion. We characterized the expression of messenger RNA that codes for a carboxyesterase in A. calandrae. (Alan K. Dowdy).

HEAT STERILIZATION PROCEDURES FOR INSECT MANAGEMENT were monitored in three processing facilities. The distribution and stratification of heat has an impact on the effectiveness of this method for insect control. Most areas examined would benefit from the addition of fans to improve air circulation that will result in more uniform application of heat. (Alan K. Dowdy).

EVALUATE THE USE OF HEAT AND DIATOMACEOUS EARTH on insect mortality. Laboratory tests indicate that the use of diatomaceous earth in combination with heat has the potential of reducing the temperature or time necessary to effect adequate insect management. When red flour beetles were treated with diatomaceous earth and exposed to 50 degree C for 15 minutes, mortality was comparable to beetles that were heated to 50 degree C for 30 minutes but not treated with diatomaceous earth. (Alan K. Dowdy).

SPATIAL ANALYSIS METHODS. The locations of stored-product insect infestations were identified using spatial analysis methods. Sources of infestations previously unknown to facility managers were identified. Insect infestations appear to be related to equipment design problems, inadequate sanitation and poor stock rotation. (Alan K. Dowdy).

USED A SPATIAL MODEL OF RUSTY GRAIN BEETLE DENSITY and bin temperature to simulate effects of time of aeration, bin size, and latitude on insect populations in stored wheat. Starting automatic aeration controllers at harvest suppressed insects below economic levels until the spring. (Paul W. Flinn/David W. Hagstrum).

MODIFIED THE MODELS IN STORED GRAIN ADVISOR (SGA) to predict effects of using automatic aeration controllers starting at harvest. SGA is presently available to the public through the extension services of Oklahoma State University, Kansas State University and Montana State University, and over 1000 copies have been distributed to customers. (Paul W. Flinn/David W. Hagstrum).

DEVELOPED A COMPUTER MODEL THAT PREDICTS THE EFFECTS OF LOW OXYGEN ATMOSPHERES on rice weevil populations in stored grain. This model can predict the duration of fumigation using low oxygen levels required to produce a given mortality and it can be used to predict insect density in grain 1-2 months post-fumigation. (Paul W. Flinn/David W. Hagstrum).

INSECT RESPONSES TO GRAIN TEMPERATURE CHANGES. Discovered that rusty grain beetles move toward warmer-temperature grain, even in very small temperature gradients. Thus, beetles will move toward the center of the grain mass as the grain cools in the fall. (Paul W. Flinn/David W. Hagstrum).

PREDICTING INSECT DENSITY FROM PROBE TRAP CATCH IN FARM-STORED WHEAT. Improved methods were developed for estimating actual insect density from trap catch using equations that adjust for the effects of grain temperature on trap catch. These methods will make probe traps more useful as monitoring tools for stored-grain insect pest management programs. (David W. Hagstrum)/Paul W. Flinn).

AREA-WIDE IPM FOR SUPPRESSION OF INSECT PESTS IN STORED WHEAT. A 5-year Area-wide IPM project was recently funded by ARS to determine whether more uniform application of insect pest management across the marketing system could reduce insect problems in stored wheat. The program should reduce the frequency of pesticide application, the cost of pest management and the risk of insect problems. (David W. Hagstrum/Paul W. Flin/ James E. Throne, Frank H. Arthur/Alan K. Dowdy/ Michael A. Mullen).

SAMPLING PROGRAMS FOR STORED-PRODUCT INSECTS. Developed a generic method for designing sampling programs for stored-product insects and demonstrated its applicability to many different types of commodity storage situations. This method will improve the quality of sampling programs and reduce the cost of developing new sampling programs. (David W. Hagstrum/Paul W. Flinn).

PARASITOID SEX PHEROMONES DISCOVERED. The parasitoids Cephalonomia tarsalis and Pteromalus cereallellae were shown to use female-produced sex pheromones to attract males of their species. (Ralph W. Howard).

INSECT RESPONSES TO BACTERIAL INVASION. Demonstrated that essential fatty acids (eicosanoids), which regulate insect immunity to bacterial infections, vary with developmental life stage. These findings suggest that control of insect pests with pathogens would be more effective if treatments were timed to occur when pest populations had their lowest level of essential fatty acids. (Ralph W. Howard).

DEMONSTRATED THAT THE ABILITY OF AN INSECT TO FIGHT BACTERIAL INFECTIONS by forming nodules is directly correlated with the insect’s age, the species of invading pathogen, and the number of pathogenic cells invading the insect’s body.

(Ralph W. Howard).

BEHAVIOR OF PARASITES OF STORED GRAIN PESTS. Demonstrated that the parasitic wasp, Cephalonomia tarsalis, has a complex behavioral pattern that it uses to locate, recognize, and paralyze its host, the saw-toothed grain beetle. This information will be used to develop improved biological control strategies that use this parasitoid. (Ralph W. Howard).

B. THURINGIENSIS SPORES AND CRYSTALS ARE NECESSARY FOR MAXIMUM INSECT TOXICITY. Spore coat protein can enhance (synergize) crystal protein and thus the combination of spores and crystals provide much more effective control of the Indianmeal moth (Plodia interpunctella) than each one can separately. (Donovan E. Johnson).

KNOWLEDGE OF NOVEL INSECT ENZYME GENES MAY LEAD TO MORE EFFECTIVE PEST MANAGEMENT. Certain enzymes that are selectively toxic to insects are being developed by ARS as biopesticides for insect control purposes. A gene for an insect molting enzyme has been cloned and characterized by ARS and Kansas State University scientists. This enzyme degrades the protective linings of the insect’s gut and exoskeleton and is toxic when fed to insects. A seed company has been licensed by ARS and KSU to evaluate this gene for resistance to pest insects when it is expressed by transgenic plants. This collaboration is a critical step in the commercial development of this transgene as a biopesticide for many types of agricultural pest insects. (Karl J. Kramer).

PATENT APPLICATIONS FOR THE USE OF INSECT CHITINASE AS A BIOPESTICIDE. ARS scientists at Manhattan, Kansas, in cooperation with scientists at Kansas State University, are working to enhance the resistance of plants to insects using chemical defense transgenes. One of the genes is an insect molting enzyme gene that can be manipulated by the agricultural biotechnology industry for the improvement of host plant resistance to insect pests. Efforts to obtain US and international patents for the use of insect chitinase as a biocide in the United States and several foreign countries are in progress. (Karl J. Kramer).

KNOWLEDGE OF INSECT SKELETAL STRUCTURES MAY LEAD TO MORE EFFECTIVE PEST MANAGEMENT. The insect exoskeleton is a good target for novel pest management strategies because of the unique insect-specific chemistry that occurs during its formation. Development of exoskeleton-targeted insect control agents has been hampered by a lack of basic knowledge about insect skeletal structure and metabolism. ARS and university scientists have identified novel metabolic reactions in insects that help to form and stabilize the exoskeleton. Inhibition of these reactions by biopesticides may be an environmentally-safe method of insect pest control. (Karl J. Kramer).

INSECT RESISTANT PACKAGING. Assisted in development of insect resistant packages for various food packaging companies which have led to significant reductions in insect related complaints. (Michael A. Mullen).

PHEROMONE TRAPS FOR STORED-GRAIN INSECTS. Continued development of monitoring systems and pheromone-baited traps for use in processing plants, warehouses, and retail outlets. (Michael A. Mullen).

DEVELOPED NEW PHEROMONE TRAP for the Indianmeal moth which can be used in public areas but be hidden from view. (Michael A. Mullen/Alan K. Dowdy).

AN ECONOMICAL SUBSTRATE ASSAY FOR PROTEINASES IN MIXTURES WAS DEVELOPED. This assay allows the quick determination of the number and types of enzymes without prior purification. (Brenda Oppert).

NEW MECHANISM OF INSECT RESISTANCE TO BIOPESTICIDE DETERMINED. A proteinase-mediated mechanism of insect resistance to the entomocidal toxins of Bacillus thuringiensis has been identified and characterized. Resistant insects lack a proteolytic enzyme that is critical for activation of the precursor forms of the toxins. This knowledge will be helpful in developing strategies for managing resistance to biopesticides in the field. (Brenda Oppert/Karl J. Kramer).

DEVELOPED A COMPUTER MODEL FOR SIMULATING THE POPULATION DYNAMICS of the almond moth. Validated model for corn, peanuts, and dried citrus pulp. (James E. Throne).

DEVELOPED THE USE OF NEAR-INFRARED REFLECTANCE SPECTROSCOPY for detection of insect larvae in grain kernels, for disinfestations of insect-infested grain, and for identification of insects. (James E. Throne/James E. Baker).

DEVELOPED AND VALIDATED A COMPUTER MODEL FOR SIMULATING POPULATION DYNAMICS OF THE PREDATOR, LYCTOCORIS CAMPESTRIS. (James E. Throne).

IDENTIFIED COMMERCIAL CORN HYBRIDS that have resistance to maize weevils. (James E. Throne).

\*A simple whole mount technique for looking at tribolium embryos:

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Introduction, Results and Discussion:

Tribolium castaneum is becoming an important model organism for comparative investigations in insect development. In addition to gene expression studies, the species is especially well suited for genetic analysis of development (Beeman et al. 1993; Sulston and Anderson 1996; Maderspacher et al. 1998). The cuticles of first instar larvae can be studied like in Drosophila. However, often it is necessary to scrutinize earlier stages of development in order to correctly interpret mutant phenotypes. Tribolium eggs contain large spherical yolk granules of high optic density. This severely hinders the microscopic analysis of whole mount specimen, because the granules diffract light such that one cannot focus through the whole depth of an egg or embryo. Therefore, morphological details of internal organs only can be visualized after sectioning, or after the yolk has been manually removed from the embryo proper. Both methods are impractical for genetic analysis where only a minority of embryos in an egg-lay expresses the mutant phenotype.

We found that embedding of Tribolium embryos in “benz mix”, a mixture of benzyl benzoate and benzyl alcohol (Westerfield 1993), makes the yolk sufficiently transparent to allow whole mount analysis (see Fig. 1). Depending on the fixation method, the optimal composition of this mixture varies between 3.0:1 to 4.3:1 (we usually use 4:1). This embedding medium can be utilized for embryos stained by immune-histochemistry. Because the embryonic tissues become fully transparent as well, a general tissue stain is necessary for visualization of all cells. Since fluorescent dyes that intercalate into DNA (Shippy el al. 1997) are washed out by the hydrophobic benz mix, we use a modified alcoholic fuchsin staining or the classical Feulgen procedure and can be applied either alone or after immune-histochemical staining. In brief, the procedure consists of (1) washing flour off the eggs with bleach, (2) fixation, (3) cracking vitellin membranes by osmotic shock, (4) alcoholic fuchsin staining and (5) embedding.

For the alcoholic fuchsin staining it is not necessary to completely remove the vitellin membranes from the embryos, which is difficult to achieve for embryos in later developmental stages, when the serosa has become attached to the vitellin membrane. Also in these older embryos, osmotic shock with methanol usually ruptures the egg case ventrally along the amnion cavity, and this slit is sufficient for penetration of staining and embedding solutions. Upon completion of the procedure, preparations can be permanently stored in standard 1.5 ml eppendorf vials, from where they are transferred to depression slides for inspection with the stereo microscope. Alternatively, they can be mounted on standard microscope slides, either in benz mix solution or in a modified medium which is made by dissolution of 9 g powdered Canada balsam in 5 ml of benz mix solution.

Materials and Methods

Fixation solution A:3 ml PBS + 0,45 ml 37% formaldehyde + 5 ml heptanes (this amount is for one batch of embryos; prepare fresh, and shake vigorously before addition of embryos to saturate the heptane phase with fixative). Fixation solution B: 4 ml 95% ethanol + 0,5 ml 100% acetic acid + 0,2 ml 37% formaldehyde. Alcoholic fuchsin solution: 100 mg pararosaniline (C.I. 42500, Sigma P-7632; there may be batch to batch variation in quality) + 16 ml 100% ethanol + 4 ml distilled water. This solution can be kept at -20 degree C. Immediately before use, add 0,2 ml of concentrated hydrochloric acid. Benz mix: 4:1 mixture of benzyl benzoate and benzyl alcohol

Flour removal, fixation and osmotic shock :

Wash eggs twice for 4 minutes in commercial bleach (Chlorox). Wash thoroughly with water and transfer to a glass vial with fixation solution A. After 45 minutes, remove the lower phase with a Pasteur pipette. Add 10 ml of methanol and shake vigorously (osmotic shock). Remove residual heptanes and add another 5 ml of methanol such that all embryos sink to the bottom. Transfer embryos to an eppendorf tube using a cut-off blue eppendorf tip. To remove all remnants of heptanes, wash three times with methanol. Embryos now can be kept at -20 degree C, or used immediately.

Alcoholic fuchsin staining :

Fix embryos again for 1 h in 1 ml of solution B (room temperature) and then wash four times (10 minutes each) with 70% ethanol (roll on wheel). Incubate for 10 minutes in 2N HC1 in a 60 degree C water bath to hydrolyze RNAs and to modify DNA for Feulgen reaction. Wash once with water and twice 70% ethanol. Remove supernatant, add 1 ml of alcoholic fuchsin solution and incubate for 30 minutes (roll on wheel). Wash repeatedly in 95% ethanol until no more red color is released by the embryos. Wash two times in 100% ethanol to dehydrate.

Embedding:

Wash once with a 1:1 mixture of benz mix and ethanol. Let embryos settle down, remove supernatant, and add 1 ml benz mix. Mix by rolling and store in the dark at room temperature.

References

Beeman R.W., Stuart J.J, Brown S.J., DenellR.E. (1993) Structure and Function of the Homeotic Gene Complex (HOM-C) in the Beetle, Tribolium Castaneum.

 Bioessays 15 : 439-444.

Kiernan J. (1990) – Histological & Histochemical Methods : Theory & Practice.

 2nd edition, Pergamon Press, p. 128-129.

Maderspacher f, Bucher G, Klingler M (1998) – Pair-rule and gap gene mutants in the

 Flour beetle Tribolium castaneum. Dev Genes Evol., in press.

Shippy T, Brown SJ, Denell RE (1996) – Confocal imaging of Tribolium castaneum embryos. Tribolium Information Bulletin 36 : 80-82.

Sulston IA, Anderson KV (1996) – Embryonic patterning mutants in

 Tribolium castaneum. Development 122 : 805-814.

Westerfield M (1993) – The zebrafish book, Eugene, Univ. of Oregon Press.

 

Tribolium embryos after alcoholic fuchsin staining, embedded in benz mix. (A, B) and (C,D) each are two focal planes of the same embryos. Embryos are oriented with anterior up. (A) Focus on the germ rudiment of a gastrulating embryo. Serosal cells are visible around the embryo proper. (B) Same embryo as in A, with focus on the serosa cells at the dorcel side of the egg (C) Ventral view of an embryo near completion of germ band growth. Thoracic segments with developing appendages are in focus. (D) Same embryo as in C with focus on the terminal segments (lower part) and head lobes.