THE IMPORTANCE OF TAXONOMY AND OF LABORATORY STUDIES ON THE BIOLOGY, NUTRITION AND PHYSIOLOGY OF INSECTS INFESTING STORED PRODUCTS

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ABSTRACT: Taxonomic distribution of stored-product insects follows a pattern in relation to the ecological factors and the physico-chemical compositions of food products. In the course of studies on their physico-chemical specificities, a micromeritic requirement and the substrate specificity of pests were discernible. Susceptibility or resistance to attack vary with the varietal characteristics of grains and post-harvest processing, which modify hardness, gelatinization and rheological characteristics.

Nutritional requirements are satisfied by the microbial associations, even on a deficient diet on which they complete their life cycle and multiply. Nutritional aberration in axenic cultures and growth promoting or inhibitory effects of fungal associates are discussed on the basis of laboratory studies on insects.

Mineral nutrition in relation to physiological and morphological specializations has been a neglected field. Experiments on the effect of salts of divalent and monovalent ions on stored-products insects and their differential effects on the physiology nutrition and biochemistry of vertebrates and invertebrates have yielded interesting results. At low concentrations teratogenic changes and termination of metamorphosis were produced in test insects with aluminium chloride, sodium chloride and calcium phosphate. The toxicological syndromes were exhibited in the supernumerary molting, histolysis and depletion of fat, sugar and glycogen from the tissues. Data on the selective toxicities of the nutritional additives such as sugars, vitamins and aminoacids and their synergistic or antagonistic effects on tricalcium phosphate toxicity to insects are presented. Elucidation of the probable mode of action in relation to comparative biochemistry has been attempted. Antagonism of ions and reversal of toxicities of tricalcium phosphate were observed. The implications of morphological, biochemical and immunological specializations of stored-product insects are discussed in relation to the development of a few selective grain protectants for practical application.

FOOD PREFERENCES OF STORED-PRODUCT INSECTS: Taxonomically and physiologically the stored-product insects occupy a distinct place as they attack and grow on products of low moisture content. High osmotic concentration, low moisture content and factors which are inimical to insect life are the ecological conditions under which stored-product insects multiply in contrast to the requirements of the agricultural pests and insect vectors. Moisture conservation of stored-product insects is a specialized trait in their physiology and morphology [1]. Even within this group of insects it is noted that not all attack all commodities.

Curiously enough the taxonomic distribution of stored-product insects follows a pattern in relation to the ecological factors and the physicochemical composition of food products. The phenomenon, to a great extent, is governed by their habit, habitat and nutritional needs.

Nutrition in its broadest sense is a bond between physiological and ecological phenomena associated with the process of natural selection and competition for food. These have led to the development of a wide nutritional spectrum and specificity in insects. Infestation in capsicum and coffee beans, their growth on tobacco and strychnine, and survival on cellulosic materials are extreme examples of such nutritional specializations of storage insects.

In spite of the same chemical composition the particle size of a commodity exhibits a definite relationship with the type of infestation. The phenomena of specificities as exhibited by *Sitophilus oryzae* on whole cereal and *Tribolium castaneum* on milled cereal are due to their micromeretic requirements of the habitats [2]. Similarly *Callosobruchus chinensis* can thrive on milled whole pulse with husk factor while it does not grow on its processed products [3].

Spiced and processed products are infested by *Stegobium panicium*. Phenolic compounds and alkaloids are required by them. Another type of specificity is exhibited by certain insects as they preferentially attack either germ, bran or endosperm [4]. The association of mycetome and internal microflora also dictate the adaptability of an insect to breed on a commodity (Table I). The sterile environment in axenic culture does not support healthy growth of these (Table II). The olfactory and gustatory factors also govern the food preferences.

The species, in general, contain volatile and non-volatile oils, proteins, fibre, starch, minerals, tannins, etc. In most cases the characteristic flavour is due to a mixture of several constituents such as alcohols, esters, phenols, resins, etc. The volatile oils of spices contain terpenes, sesquin terpenes, alcoholds, aldehydes, esters, thiocyanates, sulphides, phenols and their derivatives [5]. Their physiological actions require further investigations. Repellancy, attractancy and toxicity are manifested by spices on some insects. The biological sequence of insect, mold, mite and bacteria, and their commensalism during bio-deterioration of grain is quite common.

INFESTATION IN PROCESSED FOODS: The cereals and legumes are processed by milling, grinding, roasting, steaming and also by formulation. Many blended and chemically fortified products are being introduced in the market. With industrialization and urbanization, the demand for processed and ready-to-serve products is increasing

TABLE I. Internal Fungal Flora Isolated From Some Stored-Product Insects (Toxigenic and Non-Toxigenic Isolates On M.S.A.)

Insects	Fungi	
	Toxigenic	Non-toxigenic
Sitophilus oryzae	*A. flavus*	*A. sydowi*
	A. candidus	*A. ruber*
	A. ochraceous	*A. niger*
	A. fumigatus	*A. chevalieri*
		P. rugulosum
		Ambylosporium sp.
		Chladosporium sp.
Tribolium castaneum	*A. flavus*	*A. versicolor*
	A. candidus	*A. niger*
	A. fumigatus	*A. ruber*
	P. islandicum	*A. chevalieri*
Trogoderma granarium	*A. flavus*	*A. ruber*
	A. candidus	*A. sydowi*
	P. islandicum	*A. niger*
		A. glaucus gr.
		A. versicolor
		A. restrictus
Callosobruchus chinensis	*A. flavus*	*A. ruber*
	A. candidus	*A. sydowi*
		A. glaucus gr.
Oryzaephilus surinamensis	*A. flavus*	*A. restrictus*
	A. ochraceous	*A. terreus*
		A. glaucus gr.
		P. decumbens
		Chlodosporium sp.
Stegobium paneceum	*A. candidus*	*A. glaucus gr.*
Araecerus fasciculatus	*A. flavus*	*A. niger*
	A. candidus	*A. glaucus gr.*

even in developing countries. The traditional products are also increasingly being processed and packed in dry and dehydrated forms for efficient marketing and distribution. The world-wide emphasis on food quality and balanced nutrition, with particular reference to proteins, vitamins, and minerals in bulk foods, has led to the development of enriched products and unconventional food formulations based on cereals, oilseeds, milk powder, egg powder, fish flour and dried meat. The rapid progress of food science and technology during the last decade has also resulted in the development of dry, dehydrated, desiccated and freeze dried packed foods. This trend has opened up new ecological patterns and consequently new problems relating to food preferences, biology, bionomics and the development of insect populations [6]. The simple example is that the case hardening by steaming or parboiling of cereals and pulses and even of macaroni and paste goods, make them highly resistant to insect infestation. The enzymes of the digestive tract of insects seem to have specific roles in dissimilating constituents of cereals and pulses. The case hardening not only induces mechanical resistance to mandibular chewing but also prevents utilization of the gelatinised starch by gut enzymes of stored-products insects.

TABLE II. Effect of Surface Sterilization on Population Numbers of Insects Raised on Rice[1]

	Numbers			
	Sterilized Rice		Unsterilized Rice	
	Control Insects	Surface Sterilized Insects	Control Insects	Surface Sterilized Insects
S. oryzae				
Adults	102	20	260	220
Pupa	20	3	35	29
Larvae	22	8	32	23
Fungi/GmX10^2	51	12	75	58
Bact./GmX10^4	110	70	318	122

[1]Total numbers after 60 days of incubation and includes 20 insects released on 20 gms rice

CLIMATIC AND GEOGRAPHICAL FACTORS: The international trade of commodities has accelerated the movement and migration of stored-product insects but the climatic and edaphic factors as presented by the global zones have restricted their growth and distribution. Distinct geographical distribution of the major species has been well documented. Environmental factors in many cases are inimical to the multiplication and distribution of large number of taxonomic groups. The most typical example of the stored-product insects is that of *Trogoderma granarium*, which is restricted in its distribution to the hot-dry belt. Its multiplication is rapid even in Sudanian and Saharan zones, where the moisture content of the grain is even below 3 percent. Most of the stored-product insects are absent in normal samples of grains in these areas. Thus the adaptive changes in *T. granarium* are remarkable features for its geographical distribution. On the other hand the low temperature adaptation of *Tribolium confusum* and *Cadra* sp. has been responsible for the distribution of these pests in the temperate and cooler zones of the world.

Chemical control and fumigation have been practiced since the 12th century B.C. The development of organic pesticides turned the attention of workers to their extensive use due to their spectacular results [7]. During the last decade opinion against their indiscriminate use and extensive application built up. This has necessitated research on the biology, ecology, nutrition and physiology of stored-product insects with a view to identifying the vulnerable points in their biology and life history for evolving measures for their control. Some of the studies carried out in these areas in recent years are reviewed in this report.

NUTRITIONAL ABERRATION: The candidate chemicals which are unlikely to be toxic in the human dietary, at least at low concentrations, were examined for their effects on stored-product insects [8]. Sodium chloride, sodium bicarbonate, magnesium sulphate, iron phosphate, sodium bromide, potassium bromide, and citric acid were chosen for their effects on the growth of *T. castaneum* (Table III).

TABLE III. Percentage Inhibition Caused by Some Inorganic and Organic Salts Added to Wheat Flour at Two Rates on Populations of *T. castaneum*.

	Rate	Percentage Inhibition				
	(%)	Chlor	Bicarb	Sulph	Phosph	Citrate
Control		0	0	0	0	0
Sodium	1	47	60	49	43	37
	3	55	64	51	51	44
Potassium	1	42	55	38	43	31
	3	44	63	40	43	39
Magnesium	1	35[1]		52	40	
	3	50[1]		52	48	
Calcium	1	55	50	40	58	46
	3	55	61	43	100	51

[1] Increased population growth over control

On the basis of the responses of the insects to the candidate chemicals, it may be concluded that almost all the experimental chemicals reduced the population growth even at low concentration of 10 ppm or 0.01%. Only magnesium chloride, on the contrary, exerted beneficial effects and resulted in enhancement of population of the test insects. The population as compared to control also increased with the concentration of magnesium chloride in the diet of *T. castaneum*. Only two salts, aluminium chloride and tricalcium phosphate (TCP) at 3% concentration in wheat flour, resulted in complete inhibition of growth and development of the insects [8]. Carbonates and bicarbonates tended to delay the development and consequently reduced the population. Similar was the action of magnesium sulphate which extended the life cycle to about 120 days. Aluminium chloride, in spite of its high toxicity to *T. castaneum* is not likely to be permitted in foodstuff at the required concentration of 3% and above. The TCP not only arrested the growth totally but also indicated substantial inhibitory action at lower concentrations. Only this seems to have great possibility for application in storage of food products as it is also acceptable as a human food additive [8,9].

Insect blood fundamentally differs from that of most of the animals in ionic concentration. Tobias [10] pointed out in 1948 that the existence of insects with low sodium and high potassium content in their blood is of physiological interest. The proper functioning of the vertebrate muscle and the nerve is usually thought to depend upon a plasma sodium/potassium ratio of greater than 1, while the intracellular ratio is less than 1. The results reported by Majumder and Bano on the inhibition of insect growth and delayed breeding brought about by the addition of an excess of some cations in their diet might have been due to an ionic imbalance in the insect system and resultant physiological disturbances [8].

Roeder [11] contends that calcium concentration in insect blood has no outstanding significance. Calcium is lower in insects than in other invertebrates. Our present study has shown calcium salts are more toxic than others; but strikingly enough all calcium salts did not bring about a high degree of inhibition in insects. This presumably indicates that it is not only the calcium ion which is toxic but the companion anion also has a major share in inducing growth inhibition. The toxic effects are accompanied by a series of abnormal changes in physiology and biochemistry. The symptomatic or diagnostic changes are reflected in the supernumerary molting, loss in weight, delay in metamorphosis, mottling of the skin, depletion of the fat and histolysis of the tissues [8,9,12]. Extremely interesting results have been obtained on the interaction of TCP with additives such as organic acids, amino acids, sugars, and some inorganic salts. These results have thrown light on the probable mode of action of TCP on insects. Among acids, vitamins, sugars and inorganic salts, three distinct groups could be classified on the basis of their actions on the toxicity of TCP to insects. Potentiation or reversion index has been computed on the basis of insect populations in the treated samples (Table IV). The rest of the compounds were indifferent in action. The potentiation of toxicity by 4 salts, 3 sugars, 3 aminoacids, and 3 vitamins were very significant. In many cases salts present as impurities in TCP samples were antagonistic and reduced its toxic action on insects (Table V) [13].

Organic acids such as tartaric and citric acids reversed the action of TCP. Interestingly enough, there was potentiation of toxicity by some sugars, particularly monosaccharides. The antidotal effects of trehalose, norlucine and glutathione, and total reversion of toxicity of TCP by cholesterol and ergosterol are the most important findings in this field of insect physiology.

Our investigations have also demonstrated that increased calcium ingestion by insects caused a significant decrease in blood cholesterol and fat levels in blood. The significant lowering of weight and scanty distribution of ill defined fat within the tissue of intoxicated larvae are indicative of the aberration in energy metabolism caused by TCP [8,12,19]. Investigation using TCP labelled with ^{45}Ca or ^{32}P showed maximum accumulation of these compounds in tissues within 72 hours of ingestion. These experiments also confirmed that calcium plays a major role in shell and muscle-cuticle formation [14,15].

In this context it is interesting to note that the moulting frequency of *Emerita asiatica* was governed by sea water calcium and, further, Sitaramaiah and Krishnan have suggested a relationship between the sea water calcium concentration and the calcification

TABLE IV. Interaction of Some Food Additives on the Inhibition of Insect Populations Reared on Media Containing 1% Tricalcium Phosphate (10 mg/g.)

Additives[1]	Percentage Inhibition	Percentage Potentiation/Reversion
No additive	74	
Sodium chloride	91	-17[2]
Sodium bicarbonate	90	-16
Ferrous phosphate	89	-16
Sodium bromide	89	-16
Tartaric acid	51	+23
Citric acid	48	+26
No additive	43	
Arginine	50	-18
Tryptophan	50	-18
Phenylalanine	50	-18
Lysine	18	+14
Norlucine	3	+28
Glutathione	0	+32
Cystine	16	+15
Glutamic acid	8	+23
Thiamin	50	-18
Pyridoxine	50	-18
Nicotinic acid	16	+15
Pentothinic acid	16	+15
Biotin	16	+15
Cyanocobalamin	16	+15
p-Aminobenzoic acid	50	-18
Cholesterol	0	+32
Ergosterol	0	+32

[1]Added to TCP at 5% (0.5mg/g)
[2] - Denotes Potentiation & + Denotes Reversion

of the cuticle [16]. In the larval stage the action of TCP, even at lowest concentration in the diet, gives rise to supernumerary moulting. The exact mechanism of the absorption of calcium phosphate from the gut of the insect is not known. However, radiographic studies indicated the transfer of Ca from the diet to the insect tissues [14]. Haemolymph and muscle-cuticle complex showed high accumulation of ^{45}Ca and ^{32}P (Table VI).

Urist [17] speculated on what normal life would be in higher animals if the two vital elements bound in the bone tissue, calcium and phosphorus in the form of calcium phosphate, made a gel instead of a hard substance. Calcium ions in the blood stream are essential to the clotting mechanism and also to muscle contraction. Phosphorus metabolism is probably responsible for the superiority of the vertebrates over all other living things. Combined

TABLE V. Calcium and Phosphorus Contents of Commercial Tricalcium Phosphate Samples.

Sample[1]	Estimated Ca (mg/g)	Estimated P_2O_5 (mg/g)	Ca $/P_2O_5$	Estimated true Ca in TCP (mg/g)	Computed TCP (%)	Insect Count[2]
S_1	68.2	14.6	4.7	20.4	35.9	97
S_2	61.0	17.6	3.4	23.2	41.0	72
S_3	57.0	34.2	1.7	47.9	50.8	79
S_4	65.1	25.8	2.5	36.1	63.8	86
S_5	57.2	31.4	1.8	44.0	73.5	19
S_6	55.3	40.4	1.4	56.6	100.0	12
Control						99

[1] S_1 - S_6 are different commercial samples
[2] Insect count for 100g sample

TABLE VI. Distribution of Radioactive ^{45}Ca and ^{32}P in Tissues of *T. castaneum* Larvae Fed on a Diet Containing 2620 Counts/Min or 3360 Counts/Min Respectively.

Tissue	Radioactivity (Counts/min per mg)	
	^{45}Ca	^{32}P
Fat body	36 ± 7.0	56 ± 2.3
Muscle-Cuticle	300 ± 7.0	290 ± 6.3
Whole larvae	860 ± 14.3	905 ± 22.8

with fats, carbohydrates and proteins, phosphorus is a vital constituent of every cell. The breakdown and synthesis of glycogen would be impossible without the phosphates.

MacGregor [18] believes few will deny the existence of lipid ionic exchange between the mineral component of the skeleton and the calcium (Ca^{++}) and inorganic phosphate (P^{---}) ions of the circulating body fluids of man. He developed the suggestion that the bone mineral could be said to have a solubility product in terms of calcium $(Ca_3^{++}PO_4^{---})_2$ at physiological pH, temperature and ion strength. No information on the physico-chemical equilibria of calcium and phosphorus in insect haemolymph particularly in stored grain insects is yet available.

From the above review it is quite evident that the substances which could inhibit the growth of insects need not have general toxicity to other forms of life. The inhibitory effect of TCP belongs in this category. The interactions of sugars on the toxic effects of TCP were reported by Majumder and Bano [19], the potentiating action of glucose and the antidotal effect of trehalose appear to have far reaching implications in the search for a specific insecticidal substance. The nonreducing disaccharide, alpha-alpha trehalose, is the major sugar in the insect plasma and it is believed to play a significant role in carbohydrate transport in insects, by conveying glucose units from the fat body glycogen to the site of the metabolism in other tissues [20]. Since glucose, fructose and sucrose occur in small or trace amounts in the blood of insects and trehalose in present to the extent of 90% of the blood sugar level, the role of TCP seems to be related to the imbalance created in the energy metabolism of insects. The data on the population and histopathological aspects of insects fed on glucose - or trehalose - treated TCP have indicated such a possibility. Further, at low concentrations, the supernumerary moulting in larvae and nodular outgrowths on the pupal case in insects maintained on diets containing low concentrations of TCP throws light on the involvement of the comparative physiology and biochemistry of mammals and arthropods in the manifestation of malignancy [8,9,12,19].

Vertebrates are characterized by the endoskeleton with 99% of the body calcium bound in the hydroxyapatite crystallites of bone and teeth. Their requirement of calcium phosphate should be in sharp contrast with that of invertebrates where the endoskeleton is absent. In this context, it is worth while to mention that the cholesterol and ergosterol and the vitamins of the D-group could ameliorate toxicity of TCP to insects. These vitamins are responsible for mobilization and transport of calcium in the vertebrate system. The reversion of toxicity with vitamin-D seems to point out the differential action of TCP in insects and mammals. It is well known that the fat soluble vitamins like A,D,K, and E are absent in insects, while they are essential in vertebrates. Thus, the physiological and bio-chemical differences between mammals and arthropods seem to be implicated in the action of TCP as a metabolic poison on insects.

The implications of calcium as a structural element and also silicon in plants and lower forms of life, seem to have been overlooked in the study of evolutionary morphogenesis. The enzymic differences as related to taxonomy and the molecular basis of the evolution of the enzymes, all these have given rise to the potential differentiation in the phylogeny and ontogeny of the organisms. A role of the structural elements in the evolution of vertebrates and invertebrates is indicated in this review. The recent advances in the area of the biochemistry of supporting materials have been documented by Tracey [21].

FUTURE POSSIBILITIES: Hydrated silica, calcium carbonate, magnesium carbonate, calcium phosphate, strontium sulphate and iron oxide are amongst the inorganic salts forming the structural essential to the life of an organism and these have perhaps more immediate importance to biochemical investigation than those which appear adventitious or excretory in origin. Even in the area of insect pathology and the field of bacterial control of insects, the recent findings relate to the mode of action of *Bacillus thuringiensis* in which the parasporal body plays a pivotal role. Silicon has been discovered as the structural matrix of the rhomboidal crystal in which aminoacids form the endotoxin moiety. The toxicity of the crystalline parasporal body has been attributed to silica in changing the permeability of the paratrophic membrane of the midgut of insects. Similarly in viruses the infectivity is also partly contributed by calcium or silicon.

There is no doubt that the phylogenetic development of insects has not received due attention and almost nothing is known about the comparative biochemistry of arthropods and related phyla. Though the evolutionary morphogenesis and the biochemical evolution were simultaneous, the studies in these areas of fundamental query have been negligible. In this review some of the promising inorganic chemicals and ions showing selective toxicity to insects have been taken as examples of the possibility of devising inorganic protectants.

In the present communication an attempt is being made to cite a few promising areas of research for selective control of storage insects. The greatest weakness or vulnerable point in the morphology of insects is in their cuticular structure. Mutual solubilities of pesticides and solvents in the cuticular lipoid or wax enhances absorption and transportation of the organic pesticides to the site of action where the biochemical lesion is induced [22]. The cuticle is an integral part of the exoskeleton. Insects do not have endoskeletons and bones. In spite of the basic physiological and metabolic difference between cordates and noncordates, they have not been studied from the target control point of view. There is no doubt that the phylogenetic tree needs biochemical and physiological interpretation from this angle.

The exoskeleton of insects is composed of chitin, a glucosamine polysaccharide. It is, therefore, possible to destroy the structural integrity with a specific enzyme like the chitinase without affecting the vertebrates. Recent success of Smirnoff [23] in increasing the effectiveness of *Bacillus thuringiensis* against a resistant forest insect with chitinase has indicated its future possibilities.

Metamorphosis in many insects and their high metabolic rate are also drawbacks in insect life. Curiously enough, almost all insects concentrate magnesium from their food and the phytophagous insects contain high amounts of potassium in haemolymphs which cannot be tolerated by mammals. The rapid growth in the larval stage and the histolytic changes in the pupal stage are peculiar aspects of the insect life history. They also provide clues to their specific control.

The hormones and metabolic antagonists can play a critical role in bringing disturbances in the growth and metabolism to any of the life stages of insects and during metamorphosis. Attempts to employ juvenile hormone mimics posed the problem of an extended active period of the destructive larval stage and the vexed question of a halogenated hydrocarbon. There appears to be some promising lines of research in employing folic acid antagonists and antimetabolites for blocking cholesterol metabolism in insects. Many such examples of the structural, physiological, and immunological differences between the mammals and insects have been cited by Majumder [22] with special reference to devising specific and selective controls for insects.

The weak points in the ecology and behaviour of stored product pests need elaborate investigation. Their food habits, attractance and specificity for substrates and chemotropic responses require fresh inquiry. Intergranular space as a limiting factor for spread of infestation, the action of activated kaolinite, the molecular sieve effect of meta-hydrogen halloysite for cuticular lipid and their effectiveness as sorptive dusts attack the vulnerable points of the morphological specialization of the stored product insects; and the unique metabolic aberration brought about by the tricalcium phosphate - vitamin - glucose system which seems to attack the biochemical-physiological specialization of insects as a group; and further the pathological insult by *B. thuringiensis* with chitinase or alkaligenic salts are good enough examples to encourage basic research on the biology, nutrition and physiology of insects infesting stored products.

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