DETECTION OF INSECT INFESTATION IN STORED FOODS

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I. INTRODUCTION

Food materials of agricultural and animal origin are stored in different types of storage structures for future consumption or trade purposes. During storage, pest organisms such as birds, rodents, insects, mites, and microbes

ADVANCES IN FOOD AND NUTRITION RESEARCH VOL 49
ISSN: 1043-4526

attack these commodities. More than 600 species of beetles and 70 of moths among the insects, 355 species of mites, 40 species of rodents, and 150 species of fungi have been reported to be associated with various stored products, including food commodities (Rajendran, 2002). Annual postharvest pest losses resulting from the attack of these biological agents, world over, have been estimated at 10% (Boxall, 1991). Weight losses (dry matter loss) in the range of 0.5–17% in cereals and up to 50% in pulses have been reported (Snelson, 1987). In stored dried fish in the tropics, losses up to 50% have been reported (Proctor, 1977). Inputs in the form of people power and finances invested in the production of food commodities will go to waste unless the materials are protected from depredating agents during storage. Insect pest activity in agricultural produce may start at any stage from harvest to consumption; in some cases, the infestation occurs in the standing crop itself. Insects such as *Sitotroga cerealella* (on paddy rice), *Sitophilus zeamais, Prostephanus truncatus* (on maize), *Hypothenemus hampei* (on coffee), *Carpophilus* spp. (on dried fruits), *Caryedon serratus* (on peanuts), and *Callosobruchus chinensis* (on many pulses) commence their pest activity in the standing crop or before storage (Rossiter, 1970; Tigar *et al*., 1994). Similarly, in some of the animal food products, insect pest attack has been observed during the processing stage itself. In dried fish, damage occurring to the fish in the drying yard due to blowflies (*Chrysomya megacephala, C. albiceps, C. chloropygaputoria, C. regatis*, and *Lucilia cuprina*) is a serious problem in countries in Africa and Southeast Asia. Furthermore, in cured fish when the moisture content is high at the time of processing, other types of flies including the filth fly, *Discomyza maculipennis* (Soans and Adolf, 1971), house fly, *Musca domestica*, and the cheese skipper, *Piophila casei* (Madden *et al*., 1995) are attracted.

Insect infestation causes qualitative and quantitative losses of food commodities. Insects produce excrement and frass during their grain-boring and oviposition activities. Insects like *Cryptolestes* spp., *Trogoderma granarium*, and *Plodia interpunctella* preferentially feed on the germ that is soft and highly nutritious, rather than the hard endosperm of food grains. Insect contaminants such as excreta (uric acid), exuviae (cast skins) and dead bodies, webbing, and secretions in food commodities pose a quality-control problem for food industries. These contaminants are responsible for health risks to humans (Phillips and Burkholder, 1984). The occurrence of insect fragments in processed foods is an important quality-control problem of concern in processed food industries (Gentry *et al*., 2001). There are reports on the occurrence of various levels of insect fragments in different kinds of processed foods in Brazil (Correia *et al*., 2000; Graciano *et al*., 1998; Rodrigues *et al*., 1998; Zamboni *et al*., 1988) and Italy (Bonafaccia *et al*.,

1999; Khoury *et al*., 1996; Locatelli *et al*., 1993, 2000). Pest infestation of dried fish leads to higher levels of uric acid and bacterial counts that are unacceptable to consumers and traders (Solanki, 1985). Toxicological studies revealed that excess levels of uric acid in the diet could induce hyperuricemia with associated nephropathy in rats (Starvic *et al*., 1969). Secretions from the adults of *Tribolium* spp. and *Rhyzopertha dominica* impart unacceptable off-odors to foodstuffs. Processing and end-use qualities of food commodities are also affected by insect infestation, as are cash value and marketability of different commodities.

There are national tolerance limits such as the maximum quantity of substances including pesticides and natural or unavoidable defects (live or dead insects, insect fragments, and related contaminants) allowable in a food. For instance, in the United States, the Food and Drug Administration (FDA) has established Defect Action Levels for live insects at two insects per kilogram and insect-damaged grains at 32 kernels/100 g in food grains; in wheat flour, there is a limit of 75 insect fragments/50 g, and in macaroni and noodle products, it is 225 fragments in a 225-g sample (Jeon, 2002). On the other hand, in India, according to the Prevention of Food Adulteration Act, the uric acid level in food commodities should not exceed 100 mg/kg and the number of weevil-damaged grains should not exceed 10% by count (Anon, 2001). In countries like Canada and Australia, there is zero tolerance for insects in food grains (White, 1995), and a similar standard is followed in international trade for food grains (Fleurat-Lessard, 1997).

There has been a growing concern throughout the world about contaminants such as pests and pesticides in food commodities. Quality maintenance by way of reduction in insect contaminants to meet the requirements of International Standards Organization (ISO) standards and Hazard Analysis Critical Control Points (HACCP) is important for marketing the produce. Detection of insect infestation is, therefore, necessary (1) to ensure a supply of wholesome food to the consumers, (2) to assess effectiveness of fumigation and other pesticide treatments, and (3) to serve as an early warning for taking appropriate control measures. Any delay in detection may result in pest outbreaks, causing severe contamination of food materials and quantitative loss. In addition, the detection of insect infestation in stored food commodities or on storage premises is the foremost step in pest management in food industries (Mueller, 1998).

Detection methods applicable for commodity samples and *in situ* detection and monitoring in bulk storage and food processing facilities have been reviewed (Cotton and Wilbur, 1982; Fleurat-Lessard *et al*., 1994; Milner, 1958; Pedersen, 1992; Semple, 1992). Rajendran (1999) also studied infestation detection in stored foods and storage premises.

II. INSECT PESTS OF STORED FOODS

A. TYPES

The major insect pests attacking stored food commodities are either beetles (order: Coleoptera) or moth (order: Lepidoptera) pests; psocids (order: Psocidae) are also found in most of the stored produce (Cotton and Wilbur, 1982). Food commodities of agricultural origin are often infested by beetle pests belonging to the families Anobiidae, Anthribidae, Bruchidae, Bostrichidae, Cucujidae, Curculionidae, Nitidulidae, Ptinide, Silvanidae, and Tenebrionidae, as well as moth pests belonging to Gelechiidae and Pyralidae, whereas beetles belonging to Chrysomelidae, Cleridae, and Dermestidae attack animal food products (Rees, 1996; Sedlacek *et al*., 1996). Whole grains, milled products, and different types of processed foods are infested (Table I). The beetle pests are relatively small, with the average adult size being 3–5 mm, and they are cryptic in nature and hence go unnoticed when present in food materials in low numbers. Insect pests of stored food commodities are highly prolific in that several generations can occur in a year depending on climatic conditions and availability of food. These insects have varied food habitats and can breed on foodstuffs containing less than 2% carbohydrate (e.g., *Lasioderma serricorne* and *Tribolium confusum*), on dried fish with 20% salt (e.g., *Dermestes maculatus*), on dry fruits having up to 60% sugar (e.g., *Carpophilus* spp.), and on tree nuts with 50–70% fat (e.g., *Ephestia cautella*).

B. EFFECTS ON FOODS

Insect pest infestation causes losses in quantity and quality of food commodities and changes in chemical composition, affecting the nutritive value of the produce (Howe, 1965; Scott, 1991; Swaminathan, 1977). Insect activity also leads to contamination of the produce (Table II). The flour beetles (*Tribolium* spp.) contaminate foodstuffs with their secretions, which contain 2-ethyl 1,4-benzoquinone and 2-methyl-1,4-benzoquinone. *T. castaneum* quinone secretions not only impart off-odors to food commodities but are also considered to cause liver and spleen tumors in mice (El-Mofty *et al*., 1992). However, Hodges *et al*. (1996) demonstrated that unlike in wheat flour, the accumulation of quinone secretions of *T. castaneum* adults in rice was negligible at less than 1 ppm, and hence, they claimed that *T. castaneum* infestation in rice is not likely to be a health risk. Males of *R. dominica* secrete aggregation pheromones (dominicalures) that contribute to the characteristic sweetish or musty odor in grain infested with *R. dominica* (Khorramshahi and Burkholder, 1981). However, Seitz and Sauer (1996)

TABLE I
INSECT PESTS OF STORED FOOD COMMODITIES

Scientific name	Common name
Cereals	
Corcyra cephalonica	Rice moth
Cryptolestes spp.	Grain beetles
Ephestia cautella	Tropical warehouse moth
Liposcelis spp.	Psocids
Nemapogon granella	European grain moth
Oryzaephilus surinamensis	Saw-toothed grain beetle
Paralipsa gularis	Stored nut moth
Prostephanus truncatus[a]	Larger grain borer
Plodia interpunctella	Indian meal moth
Rhyzopertha dominica[a]	Lesser grain borer
Sitophilus granarius[a]	Granary weevil
Sitophilus oryzae[a]	Rice weevil
Sitophilus zeamais[a]	Maize weevil
Sitotroga cerealella[a]	Angoumois grain moth
Tenebrio spp.	Meal worm
Tenebroides mauritanicus	Cadelle beetle
Tribolium spp.	Flour beetles
Trogoderma granarium	Khapra beetle
Pulses	
Acanthoscelides obtectus[a]	Dried bean beetle
Callosobruchus chinensis[a]	Adzuki bean weevil
Callosobruchus maculatus[a]	Cowpea beetle
Zabrotes subfasciatus[a]	Mexican bean weevil
Oilseeds and Oilcakes	
Araecerus fasciculatus	Coffee bean weevil
Caryedon serratus[a]	Groundnut borer
Elasmolomus soriddus	Lygaeid bug
Ephestia cautella	Tropical warehouse moth
Necrobia rufipes	Red legged ham beetle
Oryzaephilus mercator	Merchant grain beetle
O. surinamensis	Saw-toothed grain beetle
Tribolium spp.	Flour beetles
Trogoderma granarium	Khapra beetle
Dry fruits and tree nuts	
Amyelois transitella	Navel orange worm
Carpophilus spp.	Dried fruit beetle
Corcyra cephalonica	Rice moth
Cydia pomonella[a]	Codling moth
Ephestia calidella	Oasis dates moth
Ephestia cautella	Tropical warehouse moth

(*continued*)

TABLE I (*continued*)

Scientific name	Common name
Ephestia figulilella	Raisin moth
Ephestia elutella	Tobacco moth
Lasioderma serricorne	Cigarette beetle
Oryzaephilus mercator	Merchant grain beetle
Oryzaephilus surinamensis	Saw-toothed grain beetle
Paralipsa gularis	Stored nut moth
Plodia interpunctella	Indian meal moth
Stegobium paniceum	Drugstore beetle
Spectrobate ceratoniae	Carob moth
Tribolium spp.	Flour beetle
Vitula edmandsae serratilinella	Dried fruit moth
Beverage crops	
Araecerus fasciculatus[a]	Coffee bean weevil
Ephestia cautella	Tropical warehouse moth
Ephestia elutella	Tobacco moth
Hypothenemus hampei[a]	Coffee berry borer
Lasioderma serricorne	Cigarette beetle
Oryzaephilus mercator	Merchant grain beetle
Ptinus tectus	Australian spider beetle
Spices	
Araecerus fasciculatus[a]	Coffee bean weevil
Lasioderma serricorne	Cigarette beetle
Stegobium paniceum	Drugstore beetle
Tribolium spp.	Flour beetles
Rhyzopertha dominica	Lesser grain borer
Ephestia cautella	Tropical warehouse moth
Plodia interpunctella	Indian meal moth
Processed foods	
Cryptolestes spp.	Grain beetles
Ephestia cautella	Tropical warehouse moth
Ephestia kuehniella	Mediterranean flour moth
Lasioderma serricorne	Cigarette beetle
Liposcelis spp.	Psocids
Oryzaephilus surinamensis	Saw-toothed grain beetle
Plodia interpunctella	Indian meal moth
Stegobium paniceum	Drugstore beetle
Tribolium spp.	Flour beetles
Tubers (potato, cassava)	
Araecerus fasciculatus[a]	Coffee bean weevil
Lasioderma serricorne	Cigarette beetle
Phthorimaea operculella[a]	Potato tuber moth
Prostephanus truncatus[a]	Larger grain borer

(*continued*)

TABLE I (*continued*)

Scientific name	Common name
Animal products	
Dermestes frischii	Hide beetle
Dermestes lardarius	Larder beetle
Dermestes maculatus	Hide beetle
Tribolium spp.	Flour beetles
Lasioderma serricorne	Cigarette beetle
Necrobia ruficollis	Red-shouldered ham beetle
Necrobia rufipes	Red-legged ham beetle
Others (Dried mushrooms)	
Othocis auriculariae	Ciid beetles
Cis asiaticus	
Cis chinensis	

[a]Complete part of their life cycle inside grain, fruit, or other commodities.

stated that the odor is actually acrid or urinous, according to grain handlers and inspectors in the United States. Insect infestation in food commodities has health implications as well. The processing, cooking quality, and organoleptic properties may also be affected in infested produce (Smith *et al*., 1971; Venkatrao *et al*., 1960b). Insects also play a significant role in the dissemination and proliferation of microorganisms including mycotoxigenic fungi in food commodities. In national and international trade, the channels of commodities that are infestation free are essential to avoid rejections.

Smith *et al*. (1971) studied the baking and taste properties of insect-infested wheat flour. Changes in color, volume, size, and loaf characteristics and unacceptable taste and off-odors in bread prepared from flour infested with *Tribolium castaneum* and *T. confusum* were observed. However, in the case of *Tenebrio molitor* and *O. surinamensis*, a similar effect was observed, only with a higher level of infestation. Flour from wheat infested with *R. dominica* showed changes in rheological properties including dough stability, dough development times, water absorption, and mixing stability; bread prepared from the flour was darker, with poor crumb characteristics, and had an unacceptable off-odor (Sanchez-Marinez *et al*., 1997). However, in studies on the effects of *Sitophilus granarius* and *R. dominica* infestation in two varieties of durum wheat, Domenichini *et al*. (1994) did not find any significant changes in the rheological features of wheat flours and the quality of semolina prepared from wheat with a lower level of infestation.

TABLE II
EFFECTS OF INSECT INFESTATION ON STORED FOOD COMMODITIES

Effect	Insect	Commodity	Reference
Chemical composition altered	*Rhyzopertha dominica*	Wheat	Saxena and Singh (1994)
	Sitophilus oryzae, Tribolium castaneum, Trogoderma granarium	Wheat	Sharma *et al.* (1979)
	Sitophilus oryzae	Milled rice	Sudhakar and Pandey (1987)
	Liposcelis paetus	Milled rice	Pike (1994)
	Trogoderma granarium, Rhyzopertha dominica	Maize, sorghum, wheat	Jood *et al.* (1992, 1993a,c, 1995, 1996)
	Tribolium castaneum	Sorghum	Pant and Susheela (1977)
	Several species	Sorghum	Arora *et al.* (1993)
	Callosobruchus maculatus	Cowpea, bombara groundnut	Emefu *et al.* (1992)
		Cowpea	Ojimelukwe and Ogwumike (1999)
		Green gram	Singh *et al.* (1982)
	Callosobruchus chinensis	Chickpea	Modgil and Mehta (1996)
		Green gram, Red gram	Modgil and Mehta (1994)
		Red gram	Daniel *et al.* (1977)
	Acanthoscelides obtectus	Pulses	Regnault-Roger *et al.* (1994)
	Corcyra cephalonica, Oryzaephilus surinamensis, Tribolium castaneum, Necrobia rufipes	Peanut	Kadkol *et al.* (1957)
	Stegobium paniceum, Lasioderma serricorne	Turmeric, coriander powder	Gunasekaran *et al.* (2003)
	Araecerus fasciculatus, Rhyzopertha dominica, Sitophilus oryzae	Cassava	Padmaja *et al.* (1994); Premkumar *et al.* (1996)
	Araecerus fasciculatus	Coffee	Narasimhan *et al.* (1972)

(*continued*)

TABLE II (*continued*)

Effect	Insect	Commodity	Reference
Changes in nutritional quality	*Trogoderma granarium*, *Rhyzopertha dominica*	Wheat	Jood and Kapoor (1992a,b); Jood *et al.* (1993b)
		Sorghum	Jood *et al.* (1993b)
	Sitophilus oryzae	Finger millet	Nirmala and Kokilavani (1980)
		Wheat, maize, sorghum	Jood and Kapoor (1992b)
	Callosobruchus maculatus	Cowpea	Ojimelukwe and Ogwumike (1999); Ojimelukwe *et al.* (1999)
	Callosobruchus chinensis	Cowpea, maize	Rajan *et al.* (1975)
Off-odors	*Rhyzopertha dominica*, *Tribolium castaneum*	Sorghum	Seitz and Sauer (1996)
Changes in end-use products/quality	*Rhyzopertha dominica*	Wheat	Sanchez-Marinez *et al.* (1997)
	Sitophilus granarius, *Rhyzopertha dominica*	Wheat	Fogliazza *et al.* (1993)
	Trogoderma granarium, *Rhyzopertha dominica*	Wheat, maize, sorghum	Jood *et al.* (1993c)
	Tribolium confusum, *Ephestia kuehniella*	Wheat flour	Fogliazza *et al.* (1993)
	Tribolium castaneum	Wheat flour	Venkatrao *et al.* (1960b)
	Tribolium confusum, *Tribolium castaneum*, *Tenebrio molitor*, *Trogoderma granarium*, *Oryzaephilus surinmensis*	Wheat flour	Smith *et al.* (1971)
	Oryzaephilus surinamensis, *Tribolium castaneum*	Wheat flour	Pagani *et al.* (1996)
	Callosobruchus maculatus	Cowpea	Ojimelukwe and Ogwumike (1999)
		Cowpea, bombara groundnut	Emefu *et al.* (1992)
	Ephestia cautella, *Corcyra cephalonica*, *Oryzaephilus surinamensis*, *Tribolium castaneum*	Peanut	Srivastava (1970)
	Caryedon serratus, *Tribolium castaneum*, *Trogoderma granarium*	Peanut	Davey *et al.* (1959)

(*continued*)

TABLE II (*continued*)

Effect	Insect	Commodity	Reference
Health implications			
1. Inhalant allergy	*Sitophilus oryzae*	Wheat (granary)	Kleine-Tebbe *et al.* (1992)
2. Ulcerative colitis	*Trogoderma glabrum*	Cereal	Gorham (1989)
3. Carcinogenic quinones	*Tribolium castaneum*	Wheat flour	EM-Mofty *et al.* (1992)
4. Association with toxigenic fungi or pathogenic microbes	*Plodia interpunctella, Ephestia cautella*	Wheat	Demianyk and Sinha (1981)
	Plodia interpunctella	Maize	Abdel-Rahman *et al.* (1969)
	Sitophilus oryzae	Maize	Ragunathan *et al.* (1974)
	Sitophilus zeamais	Maize	Dharmaputra *et al.* (1994)
	Sitophilus oryzae, Tribolium castaneum	Maize	Sinha and Sinha (1992)
	Sitophilus oryzae, Tribolium castaneum	Wheat, maize	Sinha (1994)
	Sitophilus oryzae, Rhyzopertha dominica, Tribolium castaneum	Rice	Pande *et al.* (1989)
	Ephestia kuehniella	Wheat flour	Cravedi *et al.* (1993)
	Ephestia cautella, Stegobium paniceum, Tribolium castaneum, Oryzaephilus surinamensis	Peanut, cumin, wheat flour, copra	Srinath *et al.* (1976)
	Corcyra cephalonica	Peanut	Asaf *et al.* (1977)
	Chrysomya spp.	Dried fish	Garg (1977)

Venkatrao *et al.* (1960b) noted that *T. castaneum* infestation in wheat flour resulted in deterioration in the quality of gluten; in addition, the bread prepared from infested flour had low loaf volume, poor organoleptic quality (i.e., bitter taste), and off-odor problems. Similar effects were also reported in wheat flour prepared from wheat damaged by insect pests in the field itself. Adverse effects on the processing quality of flour including slimy gluten and reduced volume of bread were caused by certain true bugs (Insecta: Pentatomidae) that feed on wheat maturing on the plant. The wheat bugs including *Eurygaster austriaca, Aelia rostrata, Chlorochroa sayi*, and *Sitodiplosis mosellana* in the United States, Europe, and Syria and *Nysius huttoni* in New Zealand damage the wheat in the developing plant by their feeding activity, and some of the proteinases in insect saliva

are believed to be responsible for poor end-use quality of the flour (Every *et al*., 1998; Hariri *et al*., 2000; Swallow and Every, 1991).

Insects are directly or indirectly associated with the occurrence of molds and increased mycotoxin levels in food commodities (Dunkel, 1998). Increased insect activity results in heating and higher moisture content, favoring mold growth. Moreover, insects themselves are involved in disseminating mold in food grains and other commodities. Higher levels of mold/mycotoxins as a consequence of increased insect activity in maize (Dharmaputra *et al*., 1994; Sinha, 1994; Sinha and Sinha, 1992), almonds (Schatzki and Ong, 2001), pistachio nuts (Doster and Michailides, 1999), and yam (Morse *et al*., 2000), and wheat have been reported (Table II).

III. DETECTION OF INSECTS IN SAMPLES

Various methods are used to detect insect infestation in commodity samples (Table III). The choice of method depends on whether the infestation is inside or outside food grains, in the surrounding premises or inside bulk grain, availability of equipment facilities, and required sensitivity. These methods are deployed for different types of food commodities in diverse storage situations, and the accuracy of detection varies. Most of the methods have the objective of detecting the presence of live insects either directly or indirectly. Free-living (external) insects are detected by visual inspection, sampling, sieving, and heat-extraction methods, whereas hidden (internal) infesters are detected by radiography, staining techniques, and near-infrared and fragment count methods. Infestation can also be detected indirectly by determining uric acid or CO_2 level. Methods for the detection of both living and dead insects are rather limited (fragment count and enzyme-linked immunosorbent assay [ELISA] methods). In general, these methods are mainly concerned about insect detection in cereals only; nevertheless, they are also useful for other commodities such as pulses and oilseeds.

A. VISUAL INSPECTION

Several clues indicate the presence of insect infestation in stored foods (Table IV). The presence of eggs of pulse beetles such as *Callosobruchus* spp. can be easily seen in infested pulses with the naked eye. Similarly, the exit holes of internal infesters such as *Sitophilus* spp., *R. dominica, Prostephanus truncatus*, and *S. cerealella* are clearly visible in infested food grains. In the case of khapra beetle (*T. granarium*) infestation, the exuviae of the larvae are indicators of the presence of the pest. Infestation by moth pests including *E. cautella, Plodia interpunctella*, and *Corcyra cephalonica* is

TABLE III
INSECT DETECTION METHODS APPLICABLE FOR COMMODITY SAMPLES

Test method	Applicability	Comments
Physical methods		
Visual inspection	Whole grains, milled products	Qualitative; only high-level infestation detected
Sampling and sieving	Whole grains, milled products	Hidden infestation not detected; commonly practiced
Heat extraction	Whole grains	Adults and larvae detected
Acoustic	Whole grains	Active stages are detected
Breeding out	Whole grains	Time consuming
Imaging techniques:		
X-ray method	Whole grains	Prohibitive capital cost
Near infrared spectroscopy	Whole grains, milled products	Rapid, expensive, can be automated
Nuclear magnetic resonance	Whole grains	Less sensitive
Chemical methods		
Serological techniques	Whole grains, milled products	Highly sensitive, species specific; shows infestation from unknown past to till date
Uric acid determination	Whole grains, milled products	Shows infestation from unknown past to till date
CO_2 analysis	Whole grains	Simple, time consuming; indicates current level of infestation; not suitable for grains having >15% moisture
Specific gravity method	Whole grains	Simple and quick; not suitable for oats and maize
Cracking and flotation method	Whole grains	Variable results noted
Fragment count	Whole grains, milled products	Highly variable results noted; shows infestation from unknown past to till date
Staining techniques:		
Egg—plugs	Whole grains	Specific for *Sitophilus* spp.
Ninhydrin method	Whole grains	Eggs and early larvae not indicated

marked by the presence of webbing or silken strands and large fecal particles in a sample of grain or grain product. Doster and Michailides (1999) reported that in stored pistachio nuts in processing plants in California, shell discoloration could be a visible indicator of infestation by the navel orange worm, *Amyelois transitella*, and associated fungal decay. Visual examination of grain for "exit holes" or "windows," which are rounded holes with smooth edges having a minimum diameter of about 0.8 mm, has

TABLE IV
VISIBLE INDICATORS OF INSECT INFESTATION IN STORED FOOD COMMODITIES[a]

Indication	Commodity	Insect species
Eggs on grain surface	Pulses (whole)	*Acanthoscelides obtectus, Callosobruchus* spp., *Zabrotes subfasciatus*
Pupal cases on shells	Peanut in shell	*Caryedon serratus*
Exit holes	Cassava	*Araecerus fasciculatus*
	Coffee seed	*Araecerus fasciculatus*
	Peanut in shell	*Caryedon serratus*
	Pulses (whole)	*Acanthoscelides obtectus, Callosobruchus* spp., *Zabrotes subfasciatus*
	Spices (whole)	*Stegobium paniceum, Lasioderma serricorne*
	Wheat, rice, maize, paddy	*Rhyzopertha dominica, Sitophilus* spp., *Sitotroga cerealella*
Shell discoloration	Pistachio nuts	*Amyelois transitella*
Silken strands present	Cereals (whole or milled)	*Corcyra cephalonica, Ephestia cautella*
	Cocoa	*Plodia interpunctella, Ephestia cautella, Ephestia elutella*
	Oilseeds, oilcakes/meals	*Ephestia cautella, Plodia interpunctella*
	Dry fruits, tree nuts	*Ephestia cautella, Ephestia calidella, Plodia interpunctella*

[a]Adapted from Rajendran (1999).

been proven as a rapid method for detecting internal infestation (Nicholson *et al.*, 1953). These workers noted that the correlation coefficient between visual examination for exit holes and cracking test (described in Section III.C.2) was 0.73. Visual examination of food grains for insect exit holes is an indirect measure of detecting internal infestation and it is a qualitative test only.

B. SAMPLING AND SIEVING

In both developing and developed countries, for insect pest detection in food grains, the sampling and sieving method is commonly practiced. The method is labor intensive and indicates the current infestation instantaneously (Hagstrum, 1994). Hidden infestation of eggs, larvae, and pupae, however, is not detected. In this simple method, representative samples are drawn from a stock of static bulk grain or grains in transit, are sieved, and are visually checked for the presence of insect pests. There are no international standards for the sampling and sieving method; yet there are national standards for use locally (Wilkin *et al.*, 1994). Some of the world's major

agencies that deal with food grains (i.e., the U.S. Federal Grain Inspection Service, the Australian Wheat Board, the Canadian Grain Commission, and the Food Corporation of India) rely on sieving and inspection for living insects in the grains to detect infestation. Various types of grain samplers are used including the bag samplers (triers), deep bin cups, pelican samplers, Ellis cup, mechanically operated vacuum probes, and grain diverters. These are used for taking grain samples (0.5–1 kg) from different kinds of storages (bag-stacks, bins, elevators) and transport containers (railcars, barges, and ships). The grain samples are sifted to recover the active free-living adults and larvae. Sieving is done either manually or mechanically depending on the number of samples to be examined or on the availability of facilities. Manual sieving is, however, very common, and the sieve mesh size varies according to the size of the grain or its product. For food grains, standard sieves of No. 10 or 12 having about 2-mm mesh size are used for separating the insects. The sampling and sieving method has been proven reliable, provided that the infestation is high at more than five insects per kilogram and when there is homogeneous distribution of insects in the grain mass (Wilkin and Fleurat-Lessard, 1991). The accuracy and reliability of grain sampling has been reported to be dependent on factors such as (1) the frequency and distribution of samples, (2) frequency and distribution of insects in the commodity, and (3) the efficiency of removal of insects from the samples (Hagstrum, 1991). It has been noted that insects such as *S. zeamais*, which are not uniformly distributed, are likely to be missed during sampling (Hodges *et al*., 1985). Tests were conducted simultaneously in France and the United Kingdom to compare the performance of two types of conventional sampling spears: a single compartment gravity spear that can collect 200-g lots and a multicompartmented (8 or 11 compartments) spear capable of collecting 300-g grain samples. Wheat samples were drawn at three depths using the spears from metal bins containing 20 tons of grains to which a known number of dead and live insects (*S. granarius* and *O. surinamensis*) had been added and checked for infestation level. It was observed that there was no significant difference between the two spear types in getting representative samples containing insects (Wilkin and Fleurat-Lessard, 1991). In experiments in the United Kingdom, wheat samples to which were added adults of *O. surinamensis* or *S. granarius* at one and five insects per 500-g sample and sieved (2-mm mesh sieve used) manually, there was complete recovery of the insects, confirming the accuracy of the method (Wilkin *et al*., 1993). White (1983) used an inclined plane sieve for pest detection in wheat grains in 30-kg samples. It was noted that by this method, it was possible to recover about 90% of *T. castaneum* and *R. dominica* added to wheat in 4–5 minutes. However, about seven runs of repeated sieving were necessary for complete recovery of *T. castaneum*, four

for *S. oryzae*, and three for *R. dominica*. The presence of dockage in the commodity delayed the recovery of the insects due to blockage of holes of 1.6 mm in the wire mesh. Pereira *et al.* (1994) compared sampling and sieving with that of a probe trap method for insect detection in 1200 tons of wheat in a silo in Brazil. Observations made every 15 days during 7 months revealed that the data from the sieve method were not consistent when compared with that of the probe trap method. The total number of insects such as *Ahasverus advena, Cryptolestes* spp., *Alphitobius* spp., *T. castaneum, Sitophilus* spp., *R. dominica*, and *Typhaea stercorea* collected by trapping was significantly higher than that obtained by the sampling and sieving method. However, it was noted that, unlike the sieve method, it was difficult to interpret the trap catch. Mechanical sieves have also been used in some of the developing countries for detecting insect pests in stored grains. A prototype machine capable of recovering insect pests occurring even at a low density of 0.2 insects/kg in 10 kg of wheat (and barley) in a short period of 1.8 minutes has been developed in the United Kingdom (Wilkin *et al.*, 1994).

The sampling and sieving method, in spite of its variable efficiency, is still popular and widely used in the trade due to time constraints and the advantage of getting results within a few minutes (Hagstrum and Subramanyam, 2000).

C. FLOTATION METHODS

Flotation methods deployed for detecting internal or hidden infestation in whole cereals and pulses are of two kinds: (1) the specific gravity method wherein a whole grain sample is directly tested with a suitable salt solution having a specific gravity less than that of the grain, and (2) the cracking-flotation technique in which whole grain is coarsely ground and treated with a mixture of an alcohol solution and light mineral oil so that the exposed insect particles, which are lighter than the grain, float on the top surface.

1. Specific gravity method

White (1957) developed the specific gravity, or densitometric, method for detecting hidden infestation in whole grains for use by grain elevator operators in the United States. This method has also been used for the detection of a dipteran, *Rhagoletis mendax* in blueberry fruit, *Vaccinum angustifolium* (Dixon and Knowlton, 1994). The larvae of stored grain insects such as *Sitophilus oryzae, S. granarius, S. zeamais, Callososbruchus* spp., and *Zabrotes subfasciatus* feed inside the grains, creating a cavity and thus reducing grain density. This difference in the density between sound (uninfested) grain kernels and infested grains is exploited for the detection of infestation

using salt solutions. Accordingly, when a mixture of uninfested and infested grains is immersed in a salt solution of appropriate density (normally 1.19 g/cm^3), for about 10 minutes, the heavier uninfested grains sink to the bottom while the lighter infested ones float. The flotation media include salt solutions like sodium silicate and sodium chloride or glycerol in water (ISO, 1987). The selection of salt solution of suitable density is very critical for infestation detection. Richter and Tchalale (1994) examined the importance of the density of the flotation medium on the efficiency of pest detection. The sensitivity and accuracy have been noted to be dependent on the use of product-adapted solutions. An adaptation coefficient, Q_a, has been suggested for preparing suitable floating media:

$$Q_a = \frac{\text{Mean density of the product}}{\text{Density of the salt solution}}$$

If the Q_a is closer to 1.0, the adaptation of the flotation medium has been found better and the results have less error. The authors tested salt solutions (sodium chloride and sodium nitrate) of different densities to detect *S. oryzae* infestation in wheat and *Zabrotes subfasciatus* in legumes, and it was noted that the optimum density of the solutions for detecting infested grain was 12–13% below the average density of uninfested grains (corresponding to a Q_a value of 1.04). The kernel density may vary even according to variety and cultivar of the grain. The suggested density values for the floating medium for wheat is 1.15; for sorghum, 1.19; and for rice and peas, 1.27 (Semple, 1992). To detect *C. chinensis* in dried peas by flotation, Hurlock (1963) used a sodium silicate solution and observed varying results when the specific gravity of the solution varied from 1.27 to 1.37. However, Somerfield (1989) detected infestation of *Bruchus pisorum* in dried peas with flotation even at a lower level of 0.5–1.0%.

The specific gravity method is a qualitative test and does not indicate the species or the specific life stage present inside the grains; it is not suitable for hulled seeds such as barley, oats, and paddy and for large-seeded grains like corn (Pedersen, 1992). The method is simple and quick and requires minimum laboratory facilities. Because of low weight, defective grains such as shriveled ones will also float with the infested grains during the test. Hence, there is a need to confirm by dissecting the floating grains and check for the presence of insects. Grains containing only eggs or early larval stages cannot be detected because there will be very little differences in the density of grains infested with these stages to make them float. Furthermore, the earliest date of pest detection in food grains will vary because the rate of feeding activity of the larvae developing inside the grain (and thereby the damage caused or cavity created) differs between species.

A densitometric separation method employing air instead of a solution has also been used to detect pest infestation (Milner *et al*., 1953). In addition to infested kernels, grains with exit holes, which are indicative of hidden infestation, are also separated.

2. *Cracking-flotation method*

The cracking-floatation procedure, an official method used in the United States (Association of Official Analytical Chemists [AOAC], 1997), involves grinding of whole grain such as wheat to a particle size of about 1.5 mm so that the internal insect stages are exposed; the coarsely ground grain is then mixed with an alcohol solution and light mineral oil to enable the insect stages and parts comprising whole adults, larvae or pupae, adult heads, larval head capsules, and cast skins from larvae, pupae, or adults to float to the surface. The latter are then collected on a filter paper and examined under a microscope (Harris *et al*., 1952). In this method, in addition to the actual number of insects, different life stages present in the sample are observed. A level of detection of larvae of 95–97% has been reported. Thind and Griffiths (1979) developed a flotation technique for the detection and determination of mites in animal feeds. The method was later modified for the determination of mite and insect contaminants in food and foodstuffs with improved sensitivity (Thind, 2000). When a mixture of industrial methylated spirit (46% by volume) and glycerol (54%) was used as flotation medium, the recovery of mites and contaminants including insect (*T. castaneum*) fragments in different types of foodstuffs (dried fruits and nuts, wheat, barley, dried milk, various types of flour, and wheat germ) was noted to be 83% and 89%, respectively.

The cracking-flotation method has been compared with other analytical techniques. In his comparative study of four analytical methods for detecting *S. granarius* in wheat at three levels of infestation over a period of 48 days, Russell (1988) noticed that the results of x-ray and cracking-flotation methods were comparable. Brader *et al*. (2002) conducted a collaborative study of the detection of insect contamination in wheat by x-ray analysis, cracking-flotation, insect fragment test, and the ELISA technique in three laboratories. Large variations in the results of the cracking-flotation method between laboratories were observed; moreover, false negatives (i.e., zero results with 10 infested kernels) have also been encountered. Xingwei *et al*. (1999) compared the flotation method with a rearing method, the ninhydrin method, the x-ray method, and the CO_2 method for the detection of hidden infestation of *S. zeamais, R. dominica*, and *S. cerealella* in wheat and rice and *C. chinensis* in mung bean. The flotation method was the least accurate in determining the insect population in the commodities.

D. FRAGMENT COUNT OR ACID HYDROLYSIS METHOD

Pest infestation in food commodities can also be detected by the presence of fragments of insects such as elytra, head capsules, mandibles, and other sclerotized parts. The fragment count method basically involves a flotation technique but in a modified way. In the flotation method as applied for whole grains, simple salt solutions are used as a floating medium, whereas in the fragment count method, a mixture of oil and aqueous phase (besides surfactants) is used and the interfering food materials are digested with acid before allowing the insect fragments to float. The filth test (AOAC, 1997) for the determination of extraneous matter like insect fragments and rat hairs in different processed foods is based on the principle of fragment count method only. The test involves digestion of a sample with an acid, wet sieving, or a defatting treatment using a detergent or solvent. Then the insect fragments and rat hairs, which are oleophilic, are separated from food particles by the attraction of the oil phase (light mineral oil in an oil–aqueous mixture). The floating fragments are trapped or filtered and examined under a microscope (Dent and Brickey, 1984). Infestation detection by the fragment count method is applicable for various types of processed foods such as flour, chocolate, and powdered spices. However, it requires a trained person to carry out the analysis (Bair and Kitto, 1992; Gentry *et al.*, 2001).

In developed countries, there are tolerance limits for insect fragments in processed foods. In the United States, the Food and Drug Administration (FDA) allows a tolerance limit (Defect Action Level) of 75 insect fragments per 50 g of wheat flour. However, the type or size of the insect fragments is not taken into consideration in the total count. Hence, in advanced countries the fragment count method is very important for the food industries and millers. The number of insect fragments present in a processed food is influenced by the method of grinding or processing that the material has undergone. In a study of insect contamination of wheat and wheat flour in 16 mills in the United States, over a period of 12 months, Harris *et al.* (1952) observed that the ratio of insect fragments in flour to whole insects in the wheat from which it was made varied from mill to mill (13.7 to 1.0). In a comparative study on the determination of insect contamination in grains, Brader *et al.* (2002) observed wide variation in results of the fragment count test as reported by three laboratories when supplied with wheat samples containing a known number of grains infested with *S. granarius*. In addition, false positives have been noticed; in the absence of infested kernels, the three laboratories observed 5–33 fragments.

In the extraction of insect fragments, the surfactants and the mixture of oil–aqueous phases vary depending on the type of foodstuffs analyzed. For certain food commodities, acid digestion followed by washing with suitable

surfactants and sieving, the fragments could be extracted. Accordingly, for the extraction of light filth comprising insect fragments and rodent hairs in different types of cheeses, Nakashima (1994) used Tergitol Anionic 4 and 1% sodium lauryl sulfate solution as surfactants for washing after digesting the sample with 5.7% HCl containing the emulsifiers Igepal CO-730 and Igepal DM-710. The recovery of added insect fragments (elytra of *T. castaneum*) was about 96% ±0.95 in the collaborative study. Similarly, insect fragments in oriental fish products containing spice were isolated by acid digestion, sieving after washing with surfactants, and extraction. About 84–89% recovery of added *T. castaneum* fragments was reported (Glaze, 1993). To detect insect fragments in grain products such as whole-wheat flour, the sample was digested in a 3% HCl solution, the residue was defatted by boiling in isopropanol and the mixture was run through a No. 230 sieve. The lighter filth containing insect fragments and rat hairs was trapped with mineral oil in a mixture of tween 80 and Na_4 EDTA in 40% isopropanol. In flour samples that had been added with insect fragments (elytra of *T. castaneum*) at the levels of 5, 15, and 30 fragments 50 g^{-1}, the recoveries of insect fragments in the tests ranged from 86.2% to 94.0% (Glaze and Bryce, 1994).

The oil–aqueous phase system has the advantage of enhancing the lifting power of the oil globules that attract and concentrate the insect fragments (and rodent hairs). The size and shape of the oil globules can influence the extraction time and the type of fragments extracted. The oil globules are typically big when a simple mineral oil–water mixture is used for trapping the fragments. When ethanol or isopropanol is added to the aqueous system, the oil globules become smaller and the extraction time becomes longer, but the numbers of smaller fragments trapped are increased (Dent and Brickey, 1984).

E. STAINING TECHNIQUES

Staining secretions (egg plugs) or body fluids of insects (hemolymph) and entry holes as a means of detecting hidden insect infestation in food commodities were considered as early as the 1950s. Staining, a chemical indicator technique, is a direct method of establishing hidden living infestation in a commodity. Staining techniques are of three types (Table V).

In the first method, mucilaginous secretions of weevils are stained with a suitable chemical compound. Weevils including *S. oryzae, S. granarius*, and *S. zeamais* attacking stored cereals deposit their eggs inside the grains and plug the holes or egg cavities with saliva. Using suitable coloring agents, the egg plugs in grains can be stained and identified. The extent of infestation in a grain sample is estimated by the number of egg plugs observed. The egg plugs are likely to be dislodged during grain handling, so there are chances of

TABLE V
DETECTION OF INSECT INFESTATION BY STAINING TECHNIQUES

Chemicals used	Color spot	Reference
Specific for weevil egg plugs in grains		
Acid fuchsin	Cherry red	Frankenfeld (1948)
Gentian violet	Purple	Goossens (1949)
Berberine sulfate	Greenish yellow	Milner *et al.* (1950a)
Specific for weevil entry holes in pulses		
Iodine-potassium iodide	Black	Frankenfeld (1948)
General infestation in whole grains		
Ninhydrin	Purple	Ashman *et al.* (1970); Dennis and Decker (1962)

recording less than the actual infestation level (Potter *et al.*, 1952). Moreover, this technique is not applicable for other internal infesters such as *R. dominica* and *S. cerealella* that oviposit outside the grain. When 0.5% acid fuchsin or 1% gentian violet is used for detecting insect egg plugs in cereals, it was noted that both grains damaged by insect feeding activity and those damaged by handling and mechanical operations that exposed endosperm also are stained by the compounds, resulting in false positives; only after careful examination can one differentiate damaged grains that are colored only at the periphery from that of egg plugs, which are stained completely (Fleurat-Lessard, 1988). To overcome this problem, Milner *et al.* (1950a) used 20% berberine sulfate, an alkaloid, as the coloring agent where the egg plugs are stained fluorescent yellow when observed under ultraviolet (UV) light of 366-nm wavelength. Reed and Harris (1953) compared the staining methods using berberine sulfate and acid fuchsin in the detection of egg plugs in wheat and corn; the acid fuchsin test proved more sensitive than the berberine sulfate test.

The second method involves staining larval entry holes in pulse seeds. In the third method, ninhydrin (triketohydrindene hydrate) has been used to react with the body fluids of insects developing inside grains. A purple spot develops when ninhydrin reacts with the free amino acids (and keto acids) present in the body fluids of insects (Dennis and Decker, 1962). Ninhydrin at a 0.3% level in acetone is impregnated into filter paper. When infested grains are crushed between the folds of ninhydrin-treated paper, the body fluids from the crushed insect bodies react with ninhydrin in the paper. Purple spots develop in the paper in 20–30 minutes at room temperature (20–25 °C), the number of spots indicating the number of insects present.

The development of color spots can be hastened if the paper is heated to 50 °C for 5–10 minutes. The intensity of the color spots developed depends on the life stage or size of the insect. The infestation level in the sample is expressed as the number of hidden insects (color spots developed) per kilogram of grain. An automated machine with a flow rate of 300 kernels/min and with the ninhydrin treated paper preheated to 120 °C for rapid color spot development was developed in the United States to detect hidden infestation in grains (Dennis and Decker, 1962). Subsequently in the United Kingdom, a smaller and portable machine known as the "Ashman-Simon infestation detector" has been developed (Ashman *et al.*, 1970); the latter has been claimed to detect 5–10% of eggs and early larvae, 40–60% of middle age larvae, and 80–90% of mature larvae in cereals. The ninhydrin staining method is simple and it needs very little training to conduct the test. The drawbacks of this technique include the following: (1) Eggs and early larvae are not detected, (2) grain having higher moisture content of 15% or more or having fungi gives a color reaction even in the absence of insect infestation, and (3) the ninhydrin-impregnated paper develops purple spots when touched by hand because of the contact of amino acids in perspiration on the fingers.

In a comparative study on detecting hidden infestations of *S. zeamais, R. dominica, S. cerealella*, and *C. chinensis* in cereals and pulses by five detection methods (rearing method, CO_2 method, flotation method, ninhydrin method, and x-ray method), Xingwei *et al.* (1999) observed the following order (from high to low) in terms of accuracy in detection: rearing method > ninhydrin method > x-ray method > flotation method, and the CO_2 method was rapid but not quantitative. However, when comparing different detection methods for dried peas infested with *C. chinensis*, Hurlock (1963) reported that the staining methods are unsuitable. With 0.002% berberine solutions, he noted that there was no yellow fluorescence of the bruchid eggs when observed under UV light. When 1% iodine in potassium iodide solution was used for staining, in addition to insect eggs, the exit holes and other damaged parts in the sample were stained black, making it difficult to differentiate. A similar problem was encountered when acid fuchsin was used as the staining medium.

F. CO_2 ANALYSIS

Howe and Oxley (1944) proposed the use of carbon dioxide (CO_2) produced in food grains and grain products as an indicator of insect infestation, particularly hidden infestation. The intergranular air in normal grain, which is free from insect infestation, contains about 0.03% CO_2. This level varies depending on the moisture content of the grain because of the metabolism

of the grain itself; the higher the moisture content, the greater the CO_2 level. But in the presence of pest activity, the CO_2 level increases more rapidly. The pests responsible for CO_2 output include insects, microorganisms, and mites. The CO_2 or respirometric method involves incubation of the grain or its product in an airtight enclosure for 24 or 48 hours at controlled temperatures (25° or 35 °C), and then estimating the intergranular CO_2 content with a suitable instrument. When the grain moisture content is less than 15% and is free from insect infestation, the production of CO_2 by 1 kg of grain in 24 hours at 35 °C will be less than 0.25%. However, when there is insect or any other pest infestation, the CO_2 level increases proportionately. A CO_2 concentration of about 0.5% or more is indicative of high-level pest activity in the sample.

The CO_2 content of the intergranular air can be measured by a gasometric method or an infrared gas analyzer. The infrared gas analyzer is relatively sensitive and can measure low-level infestation (Bruce and Street, 1974; ISO, 1987). The interpretation of the results based on CO_2 concentrations depends on whether a gasometric or infrared gas analysis is used (Table VI). When an infrared gas analyzer is used, the incubation time for CO_2 measurement can be reduced to less than 24 hours (Fleurat-Lessard, 1988). In a laboratory study, Street and Bruce (1976) showed that the presence of a single larva of *E. cautella* in stored dates could be detected by measuring CO_2 concentration with a Luft type of infrared gas analyzer. The CO_2 evolved can also be determined by a gas chromatograph with a TCD detector or interference refractometer. Among the instrumental methods of analysis

TABLE VI

PEST INFESTATION LEVELS IN FOOD GRAINS AS INTERPRETED ACCORDING TO THE METHOD OF INSTRUMENTAL ANALYSIS OF CO_2 IN 1 KG OF GRAIN AFTER INCUBATION FOR 24 HOURS[a]

Method		
Gasometric (% CO_2 v/v)[b]	Infrared (μl of CO_2/min)	Level of infestation
<0.2	<1.0	Nil or negligible
0.2	1.0	Low level
0.3–0.5	2.0–3.0	Light to moderate
0.6–0.9	4.0–6.0	Moderate to heavy
>1.0	>6.0	Heavy

[a]From ISO (1987).

[b]Applicable for wheat, peas, polished rice, small yellow maize, and similar small huskless hard grains; for others, the CO_2 concentration is multiplied by correction factors (e.g., large white maize, 1.18; barley, 1.25; and oats, 1.39).

for CO_2 in the grain samples, the infrared gas analyzer has proven to be relatively sensitive, the response is quicker, and it is easy to operate (Zisman and Calderon, 1991).

CO_2 measurement is an indirect method of detecting an existing insect infestation. The respiratory rate of insect eggs or early larval stages is negligible, so the CO_2 method is not applicable for grains having only those life stages. Also, it cannot be used for grains with moisture content exceeding 15%, because at higher moisture levels, grain alone evolves more CO_2 (Semple, 1992). The intergranular CO_2 level in an incubated sample is also affected by the absorption of the gas by the grain itself (water solubility of CO_2 is 0.76% v/v). Furthermore, the CO_2 concentration inside the enclosure/container is also dependent on its air tightness, as CO_2 has a high vapor pressure (71 atm; i.e., 54,000 mm Hg, at 30 °C), and hence, it will tend to leak. Infestation detection by CO_2 analysis is only a qualitative test and does not differentiate between CO_2 production by insects, microorganisms, or mites individually. Even among insects, the rate of production of CO_2 varies among species and between life stages of single species. In a laboratory study on wheat infested with 12 adults of *T. castaneum* and *C. ferrugineus*, separately and in mixed population in individual jars at 27.5° and 33 °C, Sinha *et al.* (1986a) observed that *T. castaneum* produced more CO_2 than *C. ferrugineus* during the experimental period. Generally, the older immature stages of insects produced more CO_2 than the adults. In *S. oryzae*, fourth instar larvae produced 0.25 μl of CO_2/min^{-1}, whereas the adults produced 0.01 μl of CO_2/min^{-1} only. Hence, the minimum number of insects required to produce a measurable amount of CO_2 or the detection threshold in grains varies (Howe and Oxley, 1952). In a laboratory study in Israel, Zisman and Calderon (1991) noted 0.15–0.30% CO_2 in 48 hours in 1 kg of wheat (11.5% moisture content) samples having one or two insects of the species *T. castaneum, S. oryzae*, and *R. dominica* at 26 °C. In a 24-hour test at 35 °C with dried peas infested with *C. chinensis*, Hurlock (1963) estimated about 4.9% of CO_2. Xingwei *et al.* (1999) determined hidden infestation in cereals and pulses using five methods and concluded that the rearing method followed by CO_2 measurement at 25 °C for 24 hours is the best.

The CO_2 analysis method is also applicable for monitoring infestation in farm grain storage bins. Detection of pests and grain quality deterioration in wheat, barley, and maize stored in airtight steel bins in Canada and the United States was accomplished by checking CO_2 levels inside bins. The insects present in the bin included *Cryptolestes* spp., *T. castaneum*, and *Plodia interpunctella*. CO_2 concentrations up to 2% were recorded in infested bins (Sinha *et al.*, 1986b); in uninfested bins, the CO_2 level was only 0.03%. CO_2 measurement is particularly suitable for the detection of the insects in food grains in transit and for grains intended for binning/loading in silos,

and it requires minimum apparatus/facilities. Furthermore, a large number of samples can be analyzed for CO_2 content simultaneously.

G. URIC ACID DETERMINATION

The excreta of stored product insects are composed primarily of uric acid. However, in some species, the presence of other purines in lesser proportions has been reported. Ammonia is found in the excreta of larvae of *Ephestia kuehniella*, urea and allantoin in *Tenebrio molitor*, and both urea and xanthin in larval excreta of *C. cephalonica* (Bursell, 1967). Uric acid has been used as an indicator of insect infestation in cereals and cereal products since the 1950s (Subrahmanyan *et al*., 1955; Venkatrao *et al*., 1957). Subsequently, several methods have been described to determine uric acid levels in infested foodstuffs (Table VII). Most of these methods are modifications of tests originally developed for analysis in clinical samples. Pachla *et al*. (1987) have reviewed the methods of uric acid determination in foodstuffs and biological fluids.

In the earliest colorimetric methods, uric acid was determined using Benedict's reagent (Benedict and Franke, 1922). In the reaction, either sodium cyanide or molybdate and arseno-tungstate reagents were used or the detection limit in these methods was low at ± 8 μg/g. It was then realized that in the colorimetric method, some of the uric acid–like substances also react with the color reagent, giving exaggerated values for uric acid. This was proven by the reaction of extract from the foodstuffs with and without uricase enzyme. The enzyme uricase specifically breaks down uric acid and the values obtained for uric acid–free sample are known as *apparent* uric acid. The "apparent" uric acid content was more in infested pulses than in cereals (Venkatrao *et al*., 1959). The apparent uric acid was deducted from the values obtained for the samples without any uricase enzyme activity ("total" uric acid), to arrive at the "actual" uric acid (Pillai *et al*., 1975). The "total" uric acid indicates excretory substances produced by insects and the metabolites released by fungi. Thus, the total uric acid content in a product depends on its insect population, mold count, and moisture damage to the food grain. In the colorimetric method, which involves the reaction between phosphomolybdic acid and uric acid, the intensity of color developed for measurement was relatively less. However, the sensitivity was better (detection limit 8 μg/g) than when sodium cyanide and arseno-tungstic acid were used for colorimetry (Majumdar and Agarwal, 1991). In addition to the low sensitivity of the colorimetric methods, there are problems such as risk when using sodium cyanide or arseno-tungstate as one of the reagents and interference due to turbidity of the extract; also, they are not useful for highly colored spices. Joshi *et al*. (1985) improved the official method of infestation detection (Bureau of Indian Standards [BIS], 1970) in food

TABLE VII
METHODS FOR DETERMINING URIC ACID IN FOODSTUFFS

Method	Commodity	Detection limit (μg/g)	Reference
I. Colorimetry	Cereals	≥8.0	Majumdar and Agarwal (1991); Subramanyan *et al.* (1955); Venkatrao *et al.* (1959)
II. Enzymatic			
A. Direct method			
a. Using UV	Cereal product	100	Farn and Smith (1963a)
	Cereal product	0.5	Sen (1968)
b. Colorimetric	Cereal product	32.0	Sen and Smith (1966)
	Cereal product	0.6	Sen and Vazquez (1969)
B. Indirect method	Spices	1.0	Brown *et al.* (1982)
III. Fluorometry	Cereal product	20.0	Holmes (1980)
	Cereals	0.1	Lamkin *et al.* (1991)
IV. HPLC			
A. UV detection	Cereal and cereal products	1.0	Wehling and Wetzel (1983)
B. Thin layer amperometric detection	Cereal products	2.0	Pachla and Kissinger (1977)
V. Thin-layer chromatography	Spices	5.0	Sengupta *et al.* (1972)
VI. Paper chromatography	Cereal product	100	Venkatrao *et al.* (1960a)
VII. Liquid chromatography	Cereal product	1.0	Wehling *et al.* (1984)

grains in India for use with floury materials and pulses. The modifications to this colorimetric method include change in wavelength for measurement at 510 nm instead of 715 nm and use of higher quantities of sodium tungstate and sulfuric acid for analysis of pulses, which are highly proteinaceous. In dried mushrooms, the uric acid is separated and extracted with an ammonium carbonate solution, purified with Sephadex G-10 and determined by spectrophotometry after adding Benedict's reagent (Mlodecki *et al.*, 1972).

Manual or automated methods involving uricase enzyme were subsequently developed for use with cereal products (Farn and Smith, 1963b; Sen and Smith, 1966). Laessig *et al.* (1972) used an autoanalyzer, an instrument deployed for determining uric acid in blood, for uric acid analysis in

food samples like flour and other cereal products and milk powder. With this method, the turbidity problem was eliminated by dialysis of the extract to separate uric acid from interfering substances. The detection limit of uric acid was 40 μg/g. A similar method with improved sensitivity (5 μg/g) that used immobilized uricase for wheat flour samples was reported by Roy and Kvenberg (1981). Brown *et al*. (1982) developed a modified enzymatic method using a glucose analyzer for highly colored whole spices. With this method, the degradation of uric acid by the enzyme uricase is monitored with an oxygen-sensitive electrode. In the enzymatic method, either UV detection or colorimetry was used. The enzymatic UV method is considered relatively simpler than the enzymatic colorimetric method (Farn and Smith, 1963a). The thin-layer chromatography (TLC) method is also specific for actual uric acid present in a sample (Thrasher and Abadie, 1978). The technique has been claimed to be particularly suitable for spices with high color content (Sengupta *et al*., 1972).

An advanced method of analysis involving high-performance liquid chromatography (HPLC) either with UV detection or with thin-layer amperometric detection was then developed with sensitivity ranging from 0.03 to 2.0 μg/g of cereal and cereal products. In the HPLC method involving paired ion chromatography, Cohen (1983) could estimate individual purines (at low level, i.e., 500 pg) including uric acid, hypoxanthine, and xanthine. Other methods, mainly useful for qualitative or confirmatory tests, include TLC (Sengupta *et al*., 1972; Thrasher and Abadie, 1978) and paper chromatography (Venkatrao *et al*., 1960a).

Insect detection by uric acid analysis has been compared with other detection methods for sensitivity and reliability. Wehling *et al*. (1984) observed positive correlation between number of insects present (*S. granarius, S. oryzae*, and *R. dominica*) and level of uric acid determined by liquid chromatography. Sen and Vazquez (1969) observed good correlation between the insect fragment count method and uric acid determination in infested flour. A good correlation between kernel damage due to insect feeding, insect fragment level, and uric acid content in wheat infested with *S. oryzae, R. dominica*, and *T. granarium* was also observed (Subramanyan *et al*., 1955). Galacci (1983) improved the semiautomated colorimetric method of Roy and Kvenberg (1981) and reported that the uric acid determination and fragment count data were comparable; it was observed that uric acid value of 600 μg/50 g of flour (12 μg/g) and 50 insect fragments per 50 g of flour sample (earlier FDA Defect Detection Level) were related. Wehling and Wetzel (1983) and Pachla *et al*. (1987) discussed the merits and drawbacks of manual colorimetry, enzymatic, and fluorometric methods of uric acid analysis in foodstuffs and favored HPLC because it can detect uric acid at the desired commercial level.

Infestation detection by uric acid determination has been considered relatively less sensitive because it requires a substantial insect population to produce a measurable amount of uric acid and it lacks reproducibility. However, sensitivity is greatly improved with the HPLC methods (Pixton, 1965; Wehling and Wetzel, 1983). Furthermore, the uric acid level in a particular sample indicates not only the existing but also the past infestation. Nevertheless, uric acid measurement as an indicator of the level of insect infestation is still followed in developing countries where infestation level is relatively higher (Joshi *et al*., 1985).

The amount of uric acid excreted insect/day has been noted to vary depending on the life stage, insect density, and their nutritional status (Farn and Smith, 1963b). In *T. castaneum*, among the life stages the rate of excretion of uric acid was highest in the larvae (18.0 ±5.5 μg/mg of body weight/day), whereas in the adults it was low at 5.9 ±1.5 μg/mg of body weight/day (Sen, 1968). The amount of uric acid produced by three stored product species developing part of their life cycle inside wheat was compared by Wehling *et al*. (1984). The highest amount of uric acid was produced by *S. granarius* (1.27 μg/g of uric acid–infested kernel/100 g of grain) followed by *S. oryzae* (0.48 μg/g infested kernel/100 g) and *R. dominica* (0.33 μg/g infested kernel/100 g). *Callosobruchus* spp. infesting pulses such as black gram and field bean excreted relatively higher quantities of uric acid (3100 and 4875 mg/100 g, respectively), whereas in sorghum infested with *S. oryzae* and wheat flour by *T. castaneum*, it was low at 700 and 160 mg/100 g, respectively, during a storage period of 5 months (Venkatrao *et al*., 1959). This was due to the higher protein/purine content of the pulses and the higher multiplication rate of *Callosobruchus* than the other insects. With some species (e.g., *R. dominica*), the amount of uric acid present in a particular grain sample also depends on handling of the grain such as cleaning or aspiration. This is because uric acid is accumulated more in the dust materials (Wehling *et al*., 1984). The proportion of distribution of uric acid either in frass or in the grains depends on the insect species (Pillai *et al*., 1975).

H. IMAGING TECHNIQUES

Internal infestation in food grains and other agricultural produce can be detected without damaging the product by employing different types of imaging techniques based on x-rays, nuclear magnetic resonance (NMR), near-infrared (NIR), and computed tomography (CT) scanning. There has been more focus and advancement on these techniques in developed countries.

1. X-ray technique

X-ray or radiographic techniques have various applications including the detection of internal damage due to "hollow heart" in potatoes, "watercore" in apples, and insect infestation in fruits and food grains (Tollner, 1993). The method had also been used for the detection of the seed weevil *Cryptorynchus mangiferae* in mango fruits (Thomas *et al*., 1995). The x-ray absorbance of a test region is known to be affected by tissue density, water content, presence of insects, or other foreign materials and effective atomic number. Any changes in these parameters in the test region exceeding a volume of 1 mm^3 are detected by the x-ray system (Tollner, 1993). The required exposure time and voltage of x-rays vary according to commodity, the degree of penetration, and the contrast required; grains having higher moisture need a higher voltage for the penetration of x-rays (Semple, 1992). Also, the denser the matter, the higher its atomic number and the greater the x-ray absorption. For grains, the x-ray exposure varies from several seconds to a few minutes. The x-ray method for the detection of hidden infestation in food grains was developed by Milner *et al*. (1950b) and is an official method in the United States (American Association of Cereal Chemists [AACC], 2000). It detects both living and dead insects; however, egg and early larval stages are not detected. In addition to the need for an expensive machine to generate x-rays and films for exposure, it requires an experienced person to operate the equipment and to interpret the radiographs. To radiograph a grain sample, about 100 g of grain is spread as a flat single layer in a nonabsorbent tray and exposed to x-rays. The x-ray technique is particularly useful for screening packed foods. In food processing facilities, it has an important role because it can check a series of samples to meet quality-control standards.

Available machines, which produce soft x-rays having relatively long wavelengths and weak transmission power, are provided with a microprocessor image analyzer, which provides a clear picture of the internal infestation in individual kernels. Unlike hard x-rays, the soft x-ray system is rapid, does not involve elaborate preparation of the samples, and can vividly distinguish living and dead insects. Soft x-ray–based detection of internal infestation of different stored grain insects in wheat, rice, and mung bean has been reported (Xingwei *et al*., 1999; Karunakaran, 2002).

Brader *et al*. (2002) examined the accuracy of determining insect contamination in wheat by x-ray analysis. It was observed that the x-ray analysis technique is one of the most accurate methods next to ELISA in detecting late instar larvae of *S. granarius* in wheat. Hurlock (1963) compared the radiographic method with that of staining techniques, flotation method, and CO_2 analysis to detect hidden infestation of *C. chinensis* in dried whole green

peas. He observed that the results with the x-ray method were consistent and the accuracy was better than that of other methods. Schatzki and Fine (1988) examined radiograms of wheat kernels infested with *S. zeamais, S. oryzae, R. dominica*, and *S. cerealella* and observed that the infestation of the respective species could be detected with 80% accuracy at 8, 7, 27, and 15 days after oviposition, respectively. False positives due to damaged kernels and the germ portion were recognized at 0.08%. To overcome the problem of expensive x-ray film radiography and time-consuming human visual inspection, an automated inspection system of machine recognition of insect damage in food grains has been developed (Keagy and Schatzki, 1993). However, the latter requires suitable software for the analysis and interpretation of the results.

Recognition of hidden weevil infestation of fourth instar larvae (26–28 days after oviposition) of *S. granarius* with an x-ray and image-processing technique in wheat has been reported. The image-processing algorithm recognized about 50% of infested kernels and the false positive was limited to 0.5% only (Keagy and Schatzki, 1993). Machine recognition generally increased with successive larval stage, from second instar to fourth instar. Unlike human visual detection, the machine recognition decreased at prepupa stage for *S. zeamais* and pupal stage in *S. granarius* and then increased again with later stages. Kim and Schatzki (2001) reported the application of x-ray imaging and single processing techniques for the detection of "pinholes" in almonds due to insect damage. Navel orange worm larvae (*Amyelois transitella*) when feeding on almonds make narrow holes of less than 1 mm in diameter. This damage, known as *pinholes*, is difficult to observe manually but is detected by x-ray imaging. In this context, an automatic method of detecting insect produced pinholes in the nuts was developed. The pinhole-damaged region appeared slightly darker than the undamaged region (in almond nuts) on x-ray film or line-scanned images and was recognized by a machine-recognition algorithm. Casasent *et al.* (2001) studied new morphological image-processing operations for inspection of pistachio nuts for insect damage and other defects. In the new system consisting of a blob-coloring algorithm, filters and watershed transforms, images of the nuts were produced irrespective of their orientation (touching and nontouching nuts).

2. *Near-infrared reflectance spectroscopy*

Near-infrared reflectance spectroscopy (NIRS) has been used in food industries for the determination of water, proteins, and oil content in food grains and other commodities (Williams and Norris, 2001). NIRS involves measurement of absorption spectra of a test material in the infrared region of 700–2500 nm. Compositional changes in the commodity are then correlated

with spectral changes by suitable statistical techniques such as partial least squares, Fourier transformations, or neural networks. The insects present in food commodities are detected by NIRS due to their hemolymph, lipids, or chitin content. Chitin is also present in fungi. Hence, interference may be encountered due to fungi during the detection of insects in stored grains and this has to be examined in detail. In fact, NIRS has also been deployed for detecting fungal contaminants in other agricultural produce.

Wilkin *et al.* (1986) showed that the presence of the flour mite, *Acarus siro*, in animal feed can be detected at a level of 10^5 mites/kg using NIRS. The authors considered that mite hemolymph was responsible for a change in the absorbance of water, resulting in a shift from 1934 nm to 1928 nm. In a preliminary investigation, Chambers *et al.* (1992a) demonstrated that NIRS could detect adults of *S. granarius, O. surinamensis, T. castaneum*, and *A. advena* in wheat. Samples of infested and uninfested kernels were scanned from 1100 to 2500 nm at 2-nm intervals and the reflectance spectra were recorded as log 1/R. The log 1/R spectra revealed increased absorption at the wavelengths of 1450 and 1940 nm, possibly due to water. Subsequently, it was reported that there were clear differences in the spectra of live and dead insects (adults of *O. surinamensis*). Furthermore, the detection of insects by NIRS was noted to be independent of their orientation during scanning (Chambers *et al.*, 1992b). Internal infestation consisting of larvae or pupae of *S. granarius* and external infestation comprising *O. surinamensis* adults in wheat was detected by NIRS (Ridgway and Chambers, 1996). The sensitivity of detecting external infestation was, however, low at 270 insects/kg of grain or more, probably because of the position of the insects within the grain sample.

Chambers and Ridgway (1996), while checking internal infestation (e.g., pupae of *S. granarius*) in single wheat kernels by NIRS, observed a decrease in absorbance (increase in reflectance) during scanning of kernels at 400–2500 nm. However, it was noted that measurement at two wavelengths (i.e., 1194 nm and 1304 nm) is adequate for detection instead of the full spectrum. Ghaedian and Wehling (1997) demonstrated that wheat kernels containing late-instar larvae of *S. granarius* could be detected by NIRS by the analysis of reflectance spectra at 1100–2500 nm; the infested kernels are distinguished based on calibration models using principal component analysis (PCA) of NIR spectra of the kernels and by calculating Mahalanobis distances. To reduce the cost of the NIRS system, the authors suggested using less expensive filter-based instruments rather than scanning monochromator instruments. Ridgway *et al.* (1999) reported the detection of *S. granarius* larvae and pupae in individual wheat kernels by NIRS in the very near-infrared region of 700–1100 nm using two wavelength models, log 1/R (982 nm) − log 1/R (1014 nm) or log 1/R (972 nm) − log 1/R (1032 nm), with 96% accuracy

in pest detection. They opined that the spectral differences between sound and infested kernels were either due to changes in starch content (decreased) for log 1/R (982 nm) − log 1/R (1014 nm) or changes in moisture content (increased) for log 1/R (972 nm) − log 1/R (1032 nm) with infestation; the loss in starch was due to insect feeding. They also expected that the cost of analysis by NIRS imaging techniques could be reduced if silicon detector–based charge coupled device (CCD) camera is used.

An automated NIRS system capable of scanning individual grains containing late-instar larvae of *S. oryzae, R. dominica*, or *S. cerealella* at the rate of 15 kernels/min has been developed in the United Kingdom (Chambers *et al*., 1998). The system was effective and could detect the infestation irrespective of the type/class of wheat, its protein content (range 11.32–16.2%) and moisture content (range 10.0–13.2%). The minimum detectable size of the insects by NIRS varied between species. As identified by x-ray analysis, the NIRS system has been shown to detect *R. dominica* as small as 1.1 mm^2 with 95% level confidence, whereas for *S. oryzae* it was 2.0 mm^2, and for *S. cerealella* 2.7 mm^2. For a particular insect species, the accuracy of detection increases as insect development proceeds. Accordingly, in *S. oryzae* the accuracy of detection of first instar larvae was 10%, second instar larvae 24%, third instar larvae 82%, fourth instar larvae 95%, and the accuracy was 100% for pupae and adults.

The detection of internal infestation of *R. dominica, S. oryzae*, and *S. cerealella in* wheat kernels by an automated NIRS system was reported by Dowell *et al*. (1998). Single kernels were scanned at the rate of 1/4 sec and the spectral data at wavelengths of 1000–1350 nm and 1500–1680 nm were recorded. The detection has been attributed to chitin in insects. Dowell *et al*. (1999) showed that NIRS could be used for identifying 11 species of stored grain insects due to the characteristic chemical composition of individual species or due to the differences in the absorption characteristics of cuticular lipids of the insects. However, the accuracy of detection varies with the species; *R. dominica* and *P. truncatus* could be differentiated with 100% accuracy; *Cryptolestes* spp., with 90% accuracy; *Sitophilus* spp., with 83% accuracy; and *Tribolium* spp., with 85% accuracy. Perez-Mendoza *et al*. (2003) demonstrated detection of *S. oryzae* insect fragments in flour by NIRS using a DA 7000 spectrometer. They noted that the accuracy of detection increased with the number of fragments in a sample (i.e., >90% accuracy at a level of 130 fragments/50 g of flour, 90% at >75 fragments/50 g and <40% accuracy for samples containing <75 fragments/50 g of flour). Burks *et al*. (2000) showed the feasibility of using NIRS to differentiate passable and defective (insect infested, moldy, sour, or dirty) dried figs that may carry infestation of *Amyelois transitella, Plodia interpunctella*, and *Drosophila melanogaster*. It was noted that it requires scanning of the

dried figs throughout the NIR region to detect insect-infested and other defective figs.

Earlier, NIRS methods involving scanning of the objects over the full or entire NIR wavelength spectrum, followed by partial least-squares regression of the data, were not fast (Ridgway and Chambers, 1999). However, later studies showed that the speed of detection could be improved and the process made less expensive by using imaging techniques with appropriate filters. Computerized image analysis or machine vision is already used for the detection of dockage or nongrain particles in wheat grain. A high-speed machine vision system of moderate cost is commercially available in developed countries. Hence, integration of NIRS with machine vision has been considered a promising approach for automation in insect pest detection. NIRS may serve as a valuable tool in pest detection, particularly for grains in transit (Ridgway and Chambers, 1999; Throne *et al.*, 2003). It has been noted that external infestation by adults and larvae of *O. surinamensis* was revealed in the visible region during NIRS imaging, whereas internal infestation in grains can be detected only with selected wavelengths in the NIR region. For the analysis of NIRS images and detecting infested grain, thresholding and linear feature detection has been considered very useful (Chambers *et al.*, 1998). In a subsequent study, Ridgway and Chambers (1998) demonstrated the detection of *S. granarius* larvae in wheat kernels by NIR imaging. It was observed that imaging at two wavelengths of 1202 nm and 1300 nm and subtraction (1200 −1300 nm) resulted in more obvious differences between normal and infested kernels instead of measuring at a single wavelength of 1202 nm. Furthermore, comparison of NIRS and x-ray methods revealed that the images of internal kernel infestation by NIRS was found not to coincide with the insect cavities detected by the x-ray technique.

Ridgway *et al.* (2001) developed an automated laboratory detection system for scanning grain for biocontaminants including insects (e.g., adults and larvae of *O. surinamensis*), rodent droppings, and ergot. The system consisted of a high-speed integrated machine vision software package used with a monochrome CCD camera and a personal computer. The scanning rate was about 3 kg of wheat in 3 minutes. For the detection of internal infestation of *S. granarius* in wheat, NIR imaging has been integrated with the machine vision system. Kernels containing internal infestation were identified by the presence of bright patches when measured at 981 nm (Chambers *et al.*, 2001). The detection rates by the NIRS imaging technique for insects in artificially prepared samples and in commercial samples were 100 and 96%, respectively (Ridgway *et al.*, 2002). Machine vision, along with pattern-recognition methodology was applied for the detection of insects in bulk wheat samples in the United States (Zayas and Flinn,

1998). Identification of insects including *R. dominica, Cryptolestes ferrugineus, O. surinamensis*, and *T. castaneum* and body parts of *R. dominica* in bulk wheat samples was done using digital imaging techniques. It was noted that the position (dorsal, ventral, or lateral) of the insects and any particle clinging to the insect affected the efficiency of detection. Thus, the diverse applications of NIRS in infestation detection have been well established.

3. *Nuclear magnetic resonance spectroscopy*

For structural analysis of compounds, organic chemists generally use nuclear magnetic resonance spectroscopy (NMRS). Street (1971) reported that larvae of *S. oryzae* within wheat grains, free-living adults, and larvae of *T. castaneum*, and adults of *S. oryzae* in the presence of wheat grain could be detected by NMRS. Later, Chambers *et al.* (1984) demonstrated that the technique can also be used for the detection of hidden infestation of *S. granarius* in wheat. When uninfested grain was scanned by NMRS, it gave a broad shallow peak, whereas an infested grain gave a sharp peak whose area was directly related to the size of the insect developing inside the grain. The sensitivity of detection, however, was very low, and hence, there has been no further progress on the application of NMRS for insect pest detection in grains or other foodstuffs.

I. SEROLOGICAL TECHNIQUES

Insect pest detection and quantification in foodstuffs and other stored products is also possible by serological techniques or immunological assays involving immunodiffusion, immuno-osmophoresis, and ELISA. Serological techniques have already been employed in entomology to identify particular species in a group of insects belonging to different families (Rotundo and Tremblay, 1980) and are routinely used in clinical diagnostic tests and in analysis of mycotoxins and pesticide residues. Now, in the food industry, these techniques have been employed for detecting and estimating hidden insect infestation (e.g., preadult stages of *Sitophilus* spp. and *R. dominica*).

Johnson *et al.* (1973) first proposed the feasibility of application of an immunoassay method for the detection of insect pest contamination in food commodities. In their studies, crude extracts of individual insects such as *D. melanogaster* (eggs and adults), *Plodia interpunctella* (larvae), *T. confusum* (larvae), *Ephestia elutella* (larvae), *S. oryzae* (adults), and *S. granarius* (adults) served as antigens for the development of appropriate antibodies in rabbits. Subsequently, immunochemical tests comprising immunodiffusion and passive hemagglutination were conducted with insect extract samples to detect the pest.

After a gap of 18 years, there is a renewed interest in the development of immunological tests for insect contamination in foods. Kitto (1991) and Quinn *et al.* (1992) reported immunoassays for insect detection in food commodities by an indirect (insect myosin) ELISA (iELISA) adopting the method of Browning *et al.* (1987); insect muscle protein (myosin) is not present in food grains and processed foods. Myosin from the field cricket *Acheta domestica* was used as the antigen to develop polyclonal antibodies for immunological detection of insect infestation. The immunosorbent assay essentially involves (1) coating the walls of a microtiter plate with rabbit polyclonal antibodies developed against the insect muscle protein myosin and (2) extract of a food grain/milled product containing the insect contaminant myosin (antigen) is added to the well so the insect material (myosin) is selectively captured by the antibodies coated on to the well walls of the plate. After washing the plant materials from the wells, a second antibody conjugated to an enzyme (horseradish peroxidase) is added. The second antibody, rabbit antimyosin immunoglobulin G (IgG) coupled to the enzyme also binds selectively to the insect material present. After washing again to remove the plant materials, substrate for the enzyme binding to the antibodies is added to the microwells. Due to the reactions, color development occurs, which is measured in an ELISA reader at a wavelength of 414 nm. The intensity of color is proportionate to myosin (or other protein content in the case of monoclonal antibody test) or insect mass content, which is correlated to the number of insects in the sample.

Because insect eggs do not contain myosin, the ELISA technique cannot detect infestation that contains only the egg stage. Johnson *et al.* (1973), however, claimed that fruit fly eggs can be detected by serological methods. The authors reported that the fruit fly eggs in fruit juices are detected by solid-phase radioimmunoassay as described by Johnson *et al.* (1971).

In insects, the myosin content is known to increase as the larva develops but decreases in the pupal stage and again increases in the adults (Kitto *et al.*, 1994; Schatzki *et al.*, 1993). In *S. granarius*, for instance, the estimated myosin content in the first instar larva, fourth instar larva, pupa, and preemergent adult were 0.08, 9.02, 1.88, and 6.57 μg/insect, respectively (Schatzki *et al.*, 1993). It is believed that there are very few differences in myosin structure between insect types, and hence, the ELISA technique is known to give reliable and reproducible results. However, a nonspecific background-level equivalent to 6.84 ±1.45 μg of myosin/50 g of wheat was observed during the determination of *S. granarius* infestation in wheat by iELISA (Schatzki *et al.*, 1993); the background level was still higher for milled products and some of the spices (Kitto *et al.*, 1994).

In milled products such as wheat flour, the high background level is due to formation of background color or nonspecific binding of plant materials

(e.g., lectins) to the carbohydrate moieties in the antibodies bound to the walls of the microwells. To overcome the problem, Quinn *et al.* (1992) adopted a double sandwich ELISA (sELISA) procedure based on the method of Martin *et al.* (1988). This technique has been reported to be more sensitive and is not much affected by the quantity of wheat flour sample taken for analysis.

It has been claimed that the immunological assays are more reliable than x-ray and fragment count methods (Kitto *et al.*, 1994; Quinn *et al.*, 1992). It has been shown that with radiography, one can detect only 50% of second instar larvae and nearly 100% of the other stages of *S. granarius*, whereas iELISA is sensitive to detect even first instar larvae having 1 μg of myosin/insect (Schatzki *et al.*, 1993). In wheat, iELISA can detect hidden infestation of 6 ±0.8 insects/50 g of grain, and that is of interest to the millers in the United States.

Because myosin is common for all types of insects, both pests and beneficial insects like *Trichogramma pretiasum, Xylochoris flavipes, Bracon hebetor*, and *Laelis pedatus* are detected by iELISA. This will unnecessarily boost the insect mass content and thereby show increased pest numbers in a sample. This is one of the disadvantages of ELISA. However, by adapting a specific method (species-specific sELISA), one can determine the presence of a particular insect. Chen and Kitto (1993) developed a species-specific ELISA to detect and quantify total and specific infestation levels in wheat. Monoclonal and polyclonal antibodies were developed against a protein called *W protein* with 59,500 Da and isoelectric point of 6.0 obtained from *S. granarius*. The monoclonal and polyclonal antibodies were used in determining the level of *S. granarius* infestation in wheat by species-specific sELISA. The myosin or iELISA uses only the polyclonal antisera and detects the total insect infestation and cannot quantify the contribution of individual species. However, in a practical situation, a grain or a milled product sample may carry more than a single insect species. In this context, hybridoma or monoclonal antibody technology was exploited to develop monoclonal antisera specific to *S. granarius* (Chen and Kitto, 1993) to conduct sELISA. In sELISA, monoclonal antibodies were used to capture antigens in the first instance for specificity and then the second polyclonal antibody conjugated to IgG horseradish peroxidase to improve sensitivity of the assay. Accordingly, the monoclonal antibody served to indicate the presence of *S. granarius* infestation while the polyclonal antibody detected the total infestation in the sELISA. Species-specific sELISA has also been developed to identify *T. granarium* (Staurt *et al.*, 1994).

Both ELISA and fragment count (see Section III.D) methods are indirect measures of the mass of insect material in a sample. With these methods, live and dead insects in the sample are not distinguished; hence, both contribute

to the level of pest contamination detected in the samples. ELISA gives an indication of the total amount of insect material accumulated over the period of storage but not specifically the infestation level present at the time of sampling. Immunoassay techniques for insect pest detection and quantification in food commodities require minimal laboratory facilities and proper training of the staff. The test normally takes 2 hours to conduct about 20 samples (Bair and Kitto, 1992). The technique is applicable for detecting the presence of insects in various foodstuffs with appropriate modifications in extraction procedures (Kitto *et al*., 1994). ELISA assay kits are already marketed in developed countries. In the United States, ELISA studies have mostly concentrated on detecting hidden infestation of *S. granarius* in wheat, because it is an important pest that generates insect fragments. However, the technique has potential to detect hidden infestation of other insects like *R. dominica* in food grains and for high-value food commodities such as scented rice (e.g., *Basmati* rice), dried fruits, and tree nuts.

Hidden infestation of *S. granarius* has been detected with other serological techniques including double immunodiffusion and immuno-osmophoresis (Germinara *et al*., 2000; Rotundo *et al*., 2000). The agar gel double immunodiffusion procedure takes relatively longer (analysis time ~5 days) and some nonspecific reactions have also been observed. The immuno-osmophoresis or electrosyneresis technique that combines electrophoresis and immunoprecipitation is rapid (analysis time ~4 hours), more sensitive, and without any interfering response by the grain. The immuno-osmophoretic technique can detect even a particular larval instar (e.g., second instar larvae of *S. granarius*) as two types of precipitation lines are formed for the early larval stages (second and third instar larvae) and mature larvae (fourth instar) as well as pupae, respectively. For the detection and differentiation of *T. granarium* from six other *Trogoderma* species including *Trogoderma variabile, Trogoderma inclusum, Trogoderma simplex, Trogoderma anthrenoides, Trogoderma glabrum*, and *Trogoderma sternale plagifer*, a monoclonal antibody (mAb 1B8)–based ELISA has been developed (Staurt *et al*., 1994). The selected antibody has shown very low cross-sensitivity to other species. The application of this technique in the presence of commodities has not been reported.

J. OTHER METHODS

1. Breeding-out or incubation method

Hidden infestation in a grain sample can be determined by incubating the grain under optimum temperature and humidity conditions of 25–30 °C and 70% RH and checking for adult emergence by sieving the sample at intervals

of 3 or 4 days until no more insects emerge. It is a slow process because the time taken to complete the life cycle varies between species and depends on temperature and grain moisture conditions (Semple, 1992). Xingwei *et al.* (1999), compared the efficacy of five methods (the rearing method, ninhydrin method, x-ray method, CO_2 method, and flotation method) for detecting hidden insect infestation of *S. granarius, R. dominica*, and *S. cerealella* in cereals (wheat and rice) and *C. chinensis* in mung bean. The authors observed that among the methods, the rearing or breeding-out method is the most accurate, but it takes a longer period of 4–6 weeks to arrive at the results.

2. *Heat extraction*

Free-living adults and larvae in grain samples can be separated with a Berlese-Tullgren funnel in which samples are exposed to dry heat of 70–75 °C. The method is time consuming and effective only when the grain moisture is 14% or less (Smith, 1977). Also, the number of insects that could be recovered from grain samples varied depending on sample size and grain moisture content. Generally, mature larvae are recovered rapidly because of their greater mobility compared with adults or young larvae, which take a longer time to leave the sample. The technique is simple and equipment is not very expensive. The Canadian Grain Commission uses the technique for detecting external infestation in grains (Wilkin *et al.*, 1994). The method is useful to extract the larvae of moth pests such as *C. cephalonica, Plodia interpunclella*, and *Ephestia* spp., although it takes a somewhat longer period (5–6 hours) for extraction (Fleurat–Lessard, 1988). Minkevich *et al.* (2002) investigated extraction of larvae of *C. ferrugineus* in wheat, barley, and corn by heat extraction. It was noted that thermostatically controlled heating at 50 °C of a thin layer of grain resulted in rapid recovery (detection) compared to extraction without a thermostat.

3. *Electrical method*

Wirtz and Shellenberger (1963) found that internal infestation in grain could be detected by an electrical method by measuring the capacitance of single kernels. The electrical capacitance of the kernel is influenced by the presence of internal infestation, kernel shape and size, and its moisture content. In this method, individual kernels were placed between a pair of small electrodes attached to a frequency-determining capacitance of a test resonant circuit. The physical movement of larvae inside the kernel causes changes in capacitance, which is recorded as the changes in the frequency of the test circuit. Electrical resistance readings for infested kernels were noted to be

lower (5 megohms) than for sound kernels (20 megohms). To reduce interferences due to kernel size, moisture content, and electrode variations, the grain samples were ground and then tested. Subsequently, very few studies have been carried out on electrical methods of infestation detection.

4. *Transparency method*

In the transparency or alkali treatment method, whole grains are boiled in sodium or potassium hydroxide solution so the seed coat and the endosperm become translucent, revealing the insect stages present inside (Apt, 1950). Transparency can also be achieved by soaking pulses or maize in a solution containing crystallized phenols, lactic acid, glycerin, and water. In this simple method, only late larvae, pupae, and adults that are about to emerge are detected (Keppel and Harris, 1953).

5. *Detection of insect phenols*

Potter and Shellenberger (1952) developed a spectrophotometric method for detecting insect material (insect phenols) in cereal products. In this method, dihydroxy phenols or 3,4-dihydroxyphenylacetic acid present in insect cuticle is detected by its reaction with 2,6-dichloroquinone-chlorimid, forming a phenol-indophenol dye, which is measured in a spectrophotometer at 580 nm. Because the method is elaborate and time consuming, it has not been pursued.

6. *Electronic nose technique*

"Electronic nose" technology has been applied in food industries to examine (1) measurement of fish freshness, (2) quality of meat, (3) ripeness of cheese and tomatoes, and (4) differentiation of coffee samples (Magan, 2001). This machine olfaction technique involves (1) detection of volatiles present in the sample using an array of electronic gas sensors, (2) conversion of the sensor signals into a readable format, and (3) software analysis of the data to characterize the odor profiles. The outputs of the sensor signals are interpreted by means of discriminant function analysis, principal component analysis, pattern-recognition algorithms, cluster analysis, or artificial neural networks. The sensors used in this technique are quartz crystal microbalance systems, surface acoustic waves, conducting polymers, or metal oxide semiconductors, which are highly sensitive to the presence of alcohols, ketones, fatty acids, and esters. The sensitivity of the sensors is affected by grain moisture content. For food grains, electronic nose technology has been studied for detecting mold contamination (Borjesson *et al.*, 1989) and the

presence of flour mite, *Acarus siro* (Ridgway *et al*., 1999). It has been reported that there is a potential for application of this technique to detect insects (insect produced odors) also in stored grains (Magan, 2001).

7. *Fluorescence method*

Ashman (1966) reported that insect or insect fragments in ground food commodities can be detected after staining with crystal violet and observing under UV light. Abels and Ludescher (2003) reported that immature stages of stored grain insect pests such as *T. castaneum, T. confusum, O. surinamensis*, and *C. cephalonica* (external infestations) could be detected without staining by their natural fluorescence, when the insects are exposed to long-wave UV light of 365 nm. The fluorescence of the insect stages has been attributed to chromophores (pterins).

8. *Polymerase chain reaction technique*

In their preliminary studies, Phillips and Zhao (2003) found that hidden infestations of *R. dominica* and *Sitophilus* spp. in food grains at a level of 1 larva/kg of grain, dead or alive, could be detected by DNA markers using polymerase chain reaction (PCR). Although they adopted standard gel electrophoresis for quantifying the PCR product, which is qualitative and time consuming, there is potential to make this molecular diagnostic technique quantifiable and rapid by following a non–gel detection method using fluorescence-tagged primers to mark the PCR products after amplification and detecting spectrofluorometrically.

IV. DETECTION IN STORAGE FACILITIES

A. VISUAL INSPECTION

In grain storage premises, when there is moderate to heavy infestation, one can observe crawling insects such as the late-stage larvae of *Ephestia* spp. (in search of suitable sites for pupation) and *C. cephalonica*, as well as adults of *Tribolium* spp., *O. surinamensis, Sitophilus* spp., and *Cryptolestes* spp. Furthermore, flying insects including the adults of *R. dominica, T. castaneum, Plodia interpunctella, Ephestia* spp., and *C. cephalonica* can be observed. Careful inspection of floor areas and wall surfaces in storage premises would reveal both crawling and flying insects. Dark areas, wall cracks, and crevices are the preferred areas for insects to hide, so examination of such places could reveal the presence of insects.

B. TRAPPING METHODS

Insects (adults) move around or fly in stored products or storage premises in response to volatiles emitted by food commodities and pheromones released by the adults. Moreover, adult and larval stages of insect pests wander at random because of their innate behavior for dispersal or when seeking pupation sites. The basic behavior of locomotory and flight activities of insects is exploited in insect traps in trapping the pests in stored food commodities and food-processing facilities (Barak *et al*., 1990). Therefore, the trapping methods are applicable only for insect stages that are active and mobile. Insect traps monitor the presence of pest infestation in food plants at an early stage, serve to forecast the risk of possible pest outbreak, and may indicate a need for timely control strategies. In addition, the traps help to avoid repeated grain sampling, which is labor intensive, time consuming, and scheduled or calendar-based control methods including fumigations or residual insecticide spray treatments, and thus reduce pesticide contamination in food commodities (Wright, 1989). Trapping methods are relatively sensitive, so infestation is often detected earlier than with conventional sampling and sieving methods in bulk-stored grain, grain stores, and warehouses (Cogan and Wakefield, 1987; Reed *et al*., 1991). Trap efficiency (i.e., "the portion of total insect population per unit volume captured during a sampling method") is influenced by environmental variables such as temperature, relative humidity, and light (Hagstrum *et al*., 1990a); in general, as the number of traps increases, so is the ability to detect insect populations present in commodities/premise. In industrially advanced countries, there has been tremendous progress in research in trapping techniques for insect detection and monitoring. The insect traps can be classified into two broad categories: physical and attractant or baited traps (Table VIII).

1. Traps without attractants

In traps without attractants (i.e., physical traps), the natural locomotory activity of the insects in search of food sources, partners for mating, or to avoid enemies or adverse environmental conditions has been exploited. Several types of physical traps have been used for infestation detection.

1. *Sticky traps*: It is a traditional practice to use a sticky surface to trap flying insects. Adhesive or sticky traps having a surface area treated with sticky substances like petroleum jelly and polybutane gel are used for detecting and trapping flying insects like *R. dominica, Ephestia* spp., *S. cerealella* and *Plodia interpunctella* (Hagstrum *et al*., 1994). A disadvantage with sticky traps is that they cannot be used in places where dust or excessive infestation is present, because the dust or insects collected can occlude the sticky surface

TABLE VIII
INSECT TRAPS FOR INFESTATION DETECTION IN BULK STORAGES AND FOOD HANDLING/PROCESSING ESTABLISHMENTS

Trap type	Applicable for					
	Beetles	Moths	Crawling insects	Flying insects	Adults	Larvae
I. Physical traps (unbaited traps)						
a. Sticky trap	*	*		*	*	*
b. Refuge trap	*		*		*	*
c. Pitfall trap	*		*		*	
d. Probe trap	*		*		*	
e. Pitfall cone trap	*		*		*	
f. Electronic grain probe	*		*		*	
g. Multiple funnel trap	*	*	*	*	*	
II. Attractant traps (baited trap)						
a. Light trap	*	*		*	*	
b. Food-baited traps involving						
1. Broken grains	*		*		*	*
2. Plant oils	*		*		*	*
3. Solvent extracts of grains	*		*		*	*
c. Pheromone traps involving						
1. Sex pheromones	*	*		*	*	
2. Aggregation pheromones	*		*	*	*	

(Phillips *et al.*, 2000). Stejskal (1995) reported that fewer *T. castaneum* insects were caught in commercially available sticky traps in the presence of food and shelter. Sticky traps with pheromone or food baits have been found to be highly efficient in locating and monitoring insect infestation in warehouses, grain storages, food establishments, and marketing channels (Rejesus and Butuason, 1989; Soderstorm *et al.*, 1987; Vick *et al.*, 1990).

2. *Refuge traps:* Corrugated paper often acts as a refuge or hiding site for most of the crawling beetle pests and for the larvae of *Ephestia* spp. that are about to pupate. This refuge-seeking behavior of insects has been exploited in refuge traps. The efficiency of the refuge traps is boosted when grain oils or pheromones are used as lures in these traps (Burkholder, 1984).

3. *Pitfall traps:* Pitfall, probe, or pitfall cone (PC) traps are physical traps that are placed at surface level or buried inside bulk grain. The pitfall trap consists of a plastic jar with a mesh screen over the top. The trap is placed inside the grain or on the surface layer of the bulk grain so that insects

like *O. surinamensis* and *Cryptolestes* spp. moving across the grain slip through the mesh into the trap. A pitfall trap may or may not have an oil-impregnated pad in the base to prevent escape of the insects (Chambers, 2003).

4. *Probe traps:* The probe trap, first developed by Loschiavo and Atkinson (1967), has been modified or redesigned by other workers. A probe trap consists of a plastic cylinder perforated with approximately 2.8- to 4.0-mm diameter holes (depending on the type of grain in which it is to be used) angled down into the body of the trap where a funnel leads the captured insects into a collecting tube, which is removable. The trap is inserted into the grain mass vertically and left for a week or more. Insects in the deep layer of the grains crawl into the holes and fall into the collecting tubes. The insects remain trapped inside until the trap is taken out of the grain and inspected. The probe trap is sensitive to insects such as *Tribolium* spp., *Cryptolestes* spp., and *O. surinamensis*, which are highly active; insects like *R. dominica* and *S. zeamais*, which do not move around actively, are least trapped. Subramanyam *et al.* (1989) showed that collection of grain debris and dockage in a probe trap while inserting and removing the trap from grain can be prevented if the holes in the trap are designed to slope upward instead of downward. A coating of Fluon (liquid Teflon) around the neck of the collecting tube in the probe trap prevents the escape of trapped insects from the trap (Cogan and Wakefield, 1987). The number of holes per trap, size or diameter of the holes, and the total entry area (squared meter) of commercially available traps varies. It has been reported that in spite of the sensitivity and simplicity of the device, probe traps are not widely used by grain storage managers in the United States because of time constraints, the need for additional labor to check the traps at regular intervals, and a lack of knowledge about interpreting the trap catch (Phillips *et al.*, 2000).

5. *Pitfall cone traps:* The pitfall cone (PC) trap combines the characteristics of both pitfall and probe traps to enable trapping of insects active at the surface level and in deeper layers of the bulk grain mass (Cogan *et al.*, 1991). The PC trap is reported to be cheaper than the probe trap but is equally as effective in the detection of *O. surinamensis, C. ferrugineus*, and *R. dominica* in grains in bins.

6. *Electronic grain probe:* Improvements have been made in probe trap techniques to automate the detection procedure. Reports from China and the United States indicate that probe traps fitted with either an infrared beam or piezoelectric sensors can be used for insect detection and monitoring in stored grains (Vick *et al.*, 1991; Wei *et al.*, 1999). Shuman *et al.* (1996) developed a detection device known as the Electronic Grain Probe Insect Counter (EGPIC), in which an infrared beam sensor has been installed in the probe, which identifies and records the insects as they slip

into the trap; the sensor output signals from the probe are continuously recorded in a computer. This has been commercialized (e.g., Stormax Insector) in Canada for monitoring infestations in grain silos, elevators, and similar bulk storages as an integral part of a stored grain management system (Shuman *et al.*, 2003). The authors stated that there is potential to discriminate or identify different insect species that have been trapped in the EGPIC.

7. *Multiple traps:* Trematerra *et al.* (1994) designed a trap consisting of a series of funnels fitted vertically one above the other that could trap or detect beetle and moth pests. In a 6-week trial in a warehouse storing cereals in Italy, the multifunnel trap trapped the flying adult beetles (*R. dominica, T. castaneum*, and *C. ferrugineus*). When the trap was tested in flour mills, the moth pests such as *E. kuehniella, Pyralis farinalis, Nemapogon granella*, and *S. cerealella* were also trapped.

8. *Others:* Ho *et al.* (1997) reported the use of a computer-aided automated monitoring system along with flight traps for *T. castaneum* in a rice mill in Singapore. In the system, flight traps were hung 6 m above floor level in the mill's storehouse, and the trapped insects were allowed to pass through a 5-m PVC tube into a specimen vial (at the bottom) resting on a top pan balance. The increase in weight due to insects trapped was recorded automatically. It has been claimed that use of such an automatic detection and monitoring system saves labor and time; the system, however, can be used only in a warehouse that is clean and dust free.

Grain temperatures and moisture content, pest population density, and the natural behavior of the insects are some of the factors influencing the trap catch. Moreover, the trap design, its location, and duration of trapping also matter in its efficacy (Fargo *et al.*, 1989; Pinniger, 1991; Wakefield and Cogan, 1999). In bulk-stored grains, and in facilities that produce and store food products, several factors influence the distribution of insects (Cox and Collins, 2002) and therefore the trap catch. In view of the multiple factors affecting the trap catch, the interpretation of trap catch is a major challenge (Chambers, 2003; Hagstrum, 1994). Therefore, it has been suggested that a combination of trapping and sampling methods is necessary for meaningful estimates of the insect population in a storage system (Pereira *et al.*, 1994; Reed *et al.*, 1991). Experiments comparing the physical traps with conventional sampling methods by several authors revealed that the physical traps may be or are more efficient in detecting infestation (Pereira *et al.*, 1994; Reed *et al.*, 1991; White *et al.*, 1990). In a study on the use of probe traps for insect populations comprising *R. dominica, C. ferrugineus, A. advena*, and *Typhae stercorea* in wheat stored in farm bins in Kansas, the United States, Hagstrum *et al.* (1998) observed that the traps could detect

the pests 15–37 days earlier than with sampling and sieving. Investigations revealed that incorporation of food attractants (e.g., kibbled carob) or pheromones enhances the efficiency of physical traps (Cogan and Wakefield, 1987; Rejesus and Butuason, 1989), so these are marketed as combination traps in developed countries. Fargo *et al.* (1994) tested two probe traps with or without the attractants (i.e., aggregation pheromones of *T. castaneum* or *S. oryzae*). They, however, did not observe any improved detection of the insects resulting from the presence of pheromones in the traps.

2. *Traps with attractants*

a. Light traps. Stored product insects can be detected (attracted) using physical means (light). Insect pests are attracted by light of wavelengths between 280 and 600 nm; long wavelength UV light of 365 nm and green light of 500–560 nm are particularly attractive (Rees, 1985). The UV light source can be either "black light" tubes or bulbs discharging mercury vapors and emitting radiation in the range of 300–500 nm, with a peak at 365 nm (for tubes) or 400 nm (for bulbs). Rees (1985) opined that stored-product insects, the moth pests in particular, are more attracted to green light than to UV radiation. The response of the insects toward an attractive light source is influenced by the insect species, age, sex, temperature, and other environmental conditions, as well as intensity of surrounding light and photoperiod. A light trap is composed of a suitable light source and a sticky surface or container to retain or catch the insects. Light traps are of two types: unidirectional and multidirectional. In food commodity storage facilities, only unidirectional traps that can be mounted on the walls or ceiling beams are used. Light traps are being used more as a control device (e.g., electrocutors) than for the detection of infestation (Gilbert, 1984).

b. Food bait traps. Food volatiles emanating from stored food commodities are highly attractive to insect pests. This has been used for the detection and monitoring of insect pests, particularly beetles in food commodities and storage premises (Phillips *et al.*, 1993; Pierce *et al.*, 1990). Food attractants are not species specific and are relatively cheaper than synthetic pheromones. However, unlike the pheromones, the radius of attraction with food baits is less, especially in the presence of food commodities stored in the premises. The food attractant traps are useful in detecting and monitoring both larvae and adults (Pinniger, 1990).

Food baits are of three kinds: (1) dry baits consisting of broken grains, (2) liquid baits comprising cereal and vegetable oils, and (3) distillates of carob (locust beans, *Ceratonia siliqua*) and other food grains. In the first type, broken grains of one or a mixed type are used in cloth, jute, or plastic

bags. These baited bags are distributed around grain stacks and on the floor in warehouses. The bags are examined after 1 or 2 weeks, and insects trapped are counted. The bait bags attract multiple insect species and the attracted insects remain inside the bags for a considerable period. The bait bags need replacement every 2 weeks because they lose their attractiveness with time. Use of bait bags in food warehouses and processing units is generally discouraged to avoid product contamination by the baits.

Food bait bags of various designs have been tested and found useful in attracting insects from stored grains (Strong, 1970). In earlier studies, broken grains of barley, wheat, sorghum, oats, corn, and coffee beans contained in either metal mesh, cheese cloth, jute, or burlap bags were studied as food baits for insect pest detection/surveys in food storage warehouses, ship holds, and so on. However, subsequent investigations proved that either brown rice alone (Hodges *et al*., 1985) or a mixture of wheat, peanuts, and kibbled carob was most attractive (Pinniger, 1991). In Indonesia, plastic bait bags having 2-mm apertures containing 60 g of brown rice proved effective in monitoring infestation in warehouses storing milled rice (Hodges *et al*., 1985). The brown rice bait bags were found to be efficient with the mixed-grain bait bags of Pinniger (1975); the former proved superior to the latter in attracting both *S. zeamais* and *R. dominica*. Brown rice bait bags were also noted to be far more effective than the conventional sampling method in detecting infestation. The efficiency of brown rice bags was further confirmed in experiments in warehouses in Jakarta, Indonesia. The bait bags attracted larvae of *E. cautella* and *C. cephalonica*, in addition to beetle pests including *T. castaneum, O. surinamensis, S. zeamais*, and *R. dominica* (Haines *et al*., 1991). Cereal or vegetable oils containing fatty acids have been used as attractants for insect pests. Laboratory studies showed that oat and corn oils attract *S. oryzae* adults, and the oils of rice, soybean, wheat germ, and corn attract *T. castaneum* adults (Phillips *et al*., 1993); seeds of oats and pumpkin and sesame oils are highly attractive to larvae of *T. granarium* (Barak, 1989). These oils are known to act as synergists when incorporated in physical or pheromone traps for insect detection and monitoring (Barak, 1989; Pinniger, 1991).

Distillates or volatiles from natural food media have also been shown to attract insects such as *R. dominica* (Dowdy *et al*., 1993) and *O. surinamensis* (Pierce *et al*., 1990) but not *P. truncatus* (Fadamiro *et al*., 1998). Hexanoic acid in the volatiles of the pods of the carob tree has been reported to be the primary attractant for *S. oryzae, S. zeamais, S. granarius, A. advena*, and *C. ferrugineus* (Obeng-Ofori, 1993; Wakefield, 1999; Yamamoto *et al*., 1976).

Despite the advantages of food attractants, no formulated food bait lures are available commercially. Unlike pheromones, food attractants are

not fully understood, and there is still scope for exploiting them in insect detection and monitoring (Chambers, 2003).

c. Pheromone traps. Insects secrete low-molecular-weight (100–200) and volatile chemical substances called *pheromones*, primarily for intraspecific communication purposes. There are two types of pheromones: (1) aggregation pheromones released by beetles like *Sitophilus* spp., *Tribolium* spp., *R. dominica*, and *Prostephanus truncatus* and (2) sex pheromones released by moth pests and some of the beetle pests (Table IX). The aggregation pheromones are attractive toward both male and female adults. Sex pheromones are relatively more effective over longer distances (8–15 m radius) than aggregation pheromones (1.5–3.0 m). Factors such as age of the

TABLE IX
PHEROMONES OF INSECT PESTS OF STORED FOOD COMMODITIES EMPLOYED IN TRAPS

Insect	Pheromone	
	Name	Compound
Aggregation pheromones		
Rhyzopertha dominica	Dominicalure-1	1-methylbutyl 2-methyl-2-pentenoate
	Dominicalure-2	1-methylbutyl 2,4-methyl-2-pentenoate
Prostephanus truncatus	Trun-call-1	1-methylethyl 2-methyl-2-pentenoate
	Trun-call-2	1-methylethyl 2,4-heptadienoate
Tribolium castaneum	Tribolure	4,8-dimethyldecenal
Sitophilus oryzae	Sitophinone	5-hydroxy-4-methyl-3-heptanone
Oryzaephilus surinamensis	Cucujolide IV	(Z,Z)-3,6-Dodecacien 11-olide
O. mercator	Cucujolide II	(Z)-3-Dodecen-11-olide
Cryptolestes ferrugineus[a]	Cucujolide I	(E,E)-4-8-Dimethyl-4,8-decadien-10-olide
Sex pheromones		
Lasioderma serricorne	Serricornin	4,6-dimethyl-7-hydroxy-3-nonanone
Stegobium paniceum	Stegobinone	2,3-dihydro-2,3,5-trimethyl-6-(1′-methyl-2′-oxobutyl-4H-pyran-4-one
Trogoderma granarium	Trogodermal	1,4-methyl-8-hexadecenal
Acanthoscelides obtectus[a]	—	Methyl (E)-2,4,5-tetradecatrienoate
Callosobruchus chinensis[a]	Erectin (callosobruchusic acid + mixture of other compounds)	(E)-3,7-dimethyl-2-octene-1,8-dioic acid; a mixture of methyl branched, long-chain hydrocarbons
Plodia interpunctella	ZETA	(Z,E)-9,12-tetradecadienyl acetate
Sitotroga cerealella	HDA	Z,E,7-11-hexadecadienyl acetate
Ephestia spp.	TDA	(Z,E)-9,12-tetradecadien-1-ol-acetate

[a]The pheromones for the species are yet to be commercialized.

individual, photoperiod, and temperature influence the behavioral response of insects to pheromones and these factors can affect the trap catch (Burkholder, 1984; Pinniger, 1991). Pheromones of most of the insect pests of stored food commodities have been identified, and for at least 22 species, pheromones have been developed commercially for use in traps for detection, monitoring, and control (Phillips *et al*., 2000; Plarre and Vanderwel, 1999). It has been reported that in addition to chemical synthesis, the pheromones could be produced biosynthetically in plants using appropriate precursors (Hick *et al*., 1997), so there is potential for availability of pheromones of other insects for commercial use. The pheromones, along with special additives, are impregnated into an absorbent rubber, plastic matrix, or slow-release membranes for use in the traps. Pheromones are released from the traps at a constant rate to remain effective for a long period. Traps contain replaceable adhesive areas to which insects stick when they are lured inside by the pheromone. The active period of the pheromone lure lasts about 4–12 weeks depending on the brand, the sensitivity of pheromone, and the type of packing of the pheromone lure (Anon, 2003). The bait needs regular replacement.

To detect and monitor moth pests, pheromone traps are used at an optimum height of 2–3 m and at 14-m intervals. The required trap density varies according to the pest to be detected and monitored (Rees, 1999a). The orientation of the insects to the traps is known to be mediated by chemical and visual cues or stimuli. Hence, the design, color, and pattern of the trap and the trap location are important relative to efficacy (Mullen *et al*., 1998). Traps with darker stripes on white backgrounds are generally more attractive to moths, and the moths are active in dim light rather than in daylight or in complete darkness (Quartey and Coaker, 1993).

Sex pheromone traps are an effective means of detecting and monitoring moth pests of stored food commodities in warehouses and food industries (Bowditch and Madden, 1996; Campbell *et al*., 2002; Rees, 1999a,b; Vick *et al*., 1986). Successful use of traps in cocoa consignments in transit (Mabbat, 1995), in raisin marketing channels (Soderstorm *et al*., 1987), and in flour mills (Loi *et al*., 1987) has also been reported. Pheromone traps have also been deployed for detecting beetle pests such as *Tribolium* spp. (a crawling insect) and *Lasioderma serricorne* (an active flier) in finished product warehouses and food plants (Arbogast and Mankin, 1999). Unlike moth pests, detailed studies on the use of pheromone traps for beetle pests are very limited, except for *Prostephanus tuncatus*, which infests cassava and maize in Africa (Dandy *et al*., 1991). For insects such as, *Ahasverus advena, Acanthoscelides obtactus, Callosobruchus analis, C. maculatus, C. chinensis, Oryzaephilus surinamensis, O. mercator, S. granarius, C. cephalonica, Tenebrio molitor*, and *C. ferrugineus*, the pheromones have already been identified,

but because of difficulties in synthesizing the compounds and/or lack of market demand, they are not commercially available (Chambers, 2003; Phillips, 1997; Phillips *et al*., 2000).

There are two ways to improve pest detection by pheromone traps: (1) use of pheromone blends and (2) use with food lures. Pheromone blends have proven effective in the detection of *O. surinamensis* (Boden *et al*., 1997). A combination of pheromone and food-bait attractants can enhance the trap catch, especially for species for which synthesis of pheromones is difficult or expensive. Some of the pheromones (traps) are cross-attractant (Rejesus and Butuason, 1989). "ZETA" (Table IX) released by *P. interpunctella* females is also attractive to other phycitine moths such as *E. cautella, E. figulilella*, and *E. kuehniella*. Yet in commercial traps in addition to ZETA, Z-9-tetradecanyl acetate has been used as a synergist for the detection of *Ephestia* spp. Dominicalures 1 and 2 of *R. dominica* are highly attractive to *Prostephanus truncatus* and hence were used for monitoring the latter in East Africa (Hodges *et al*., 1983). Cox and Collins (2002) have given a list of references on cross-species attraction in pheromones of beetle pests of stored products. They also stressed the need for further studies on the use of multispecies pheromone lures in traps for stored product insects. Dowdy and Mullen (1998) deployed aggregation pheromones of *R. dominica, T. castaneum*, and sex pheromone of *Trogoderma variabile* in combination with two pheromones in the same pitfall trap and examined the efficacy of trapping. It was noted that pheromones of *R. dominica, T. castaneum*, or *T. variabile* in the same trap could be used for trapping several species. Using pheromones of two or more insect species in the same trap is advantageous because it can reduce the labor and cost of detection/monitoring of different pests occurring in the same premises or commodities. Boden *et al*. (1997) developed multicomponent lures comprising macrolide lactones (cucujolides) and a fungal volatile 1-octan-3ol for cucujids such as *O. surinamensis, O. mercator, Cryptolestes turcicus, C. pusillus*, and *C. ferrugineus*, and they stated that there is potential for application of multiple component lures for other insects as well.

C. ACOUSTIC METHOD

Insects developing inside or outside food grains or other stored food commodities produce different types of sounds/noises, vibrations, and ultrasonic signals that can be measured by suitable means (Table X). Brain (1924) suggested that internal infestation in fruits and food grains can be detected by amplifying feeding and movement sounds of the insect larvae inside. Adams *et al*. (1953) showed that the immature stages of *S. oryzae* in wheat could be detected by their feeding noise. They also put forth the idea of

TABLE X
SOUNDS/VIBRATIONS PRODUCED BY STORAGE INSECT PESTS

Type of sound/vibration	Life stage	Species	Commodity
Feeding noise	Larvae	*Sitophilus oryzae, R. dominica, Sitotroga cerealella*	Cereals
		Drosophila spp.	Grapefruit, mangoes
Probing for oviposition	Adults	*Sitophilus oryzae, S. zeamais, S. granarius*	Cereals
Boring activity	Adults	*R. dominica*	Cereals
		Prostephanus truncatus	Maize
Ultrasonic signals	Larvae	*Callosobruchus* spp.	Pulses
Mechanical vibrations due to movement	Adults and larvae	*S. oryzae, S. zeamais, S. granarius, T. castaneum*	Cereals
Ultrasonic pulses due to calling behavior	Adults	*Corcyra cephalonica, Ephestia* spp., *Plodia interpunctella*	Cereals

acoustic detection in grain stored in elevators, bins, and silos by installing sensor cables similar to thermocouple systems that are used for monitoring grain temperature. Bailey and McCabe (1965) directly placed individual wheat grains containing the larvae of *S. granarius* on a transducer and mechanical vibrations caused by the feeding activity of the larvae were amplified and the signals fed to a display system to detect the infestation. After nearly 2 decades, there has been renewed interest in acoustic detection techniques for application to grain samples and for *in situ* detection and monitoring in bulk storage in bins and elevators. Initially, the acoustic method was aimed at detecting internal infestation in food grains. However, later studies with advanced equipment have shown that the technique can be used to detect insect stages developing outside the grains as well. Hagstrum (1991) has reviewed the acoustic detection methods.

In earlier studies, microphones and phonograph cartridges were used to detect insect feeding noises in individual grains (Adams *et al*., 1953; Bailey and McCabe, 1965). Later, high-frequency (ultrasonic signals of 40 kHz) detectors were used. Acoustic emissions (sound waves) with frequencies in the ultrasonic range exceeding 20 kHz (the upper limits of human hearing) are said to be generated by microfractures in materials because of characteristic biting of grain tissue by insect larvae (Shade *et al*., 1990). In the detector system, the electrical signals produced by the ultrasonic transducer in response to the feeding sounds are amplified, demodulated, and then displayed on a loud speaker or on a strip chart recorder. The ultrasonic device used in the system detects only biting activity but not other movements and has

the advantage that there is no background interference, so there is no need for noise shielding. Unlike other acoustic systems wherein the low-frequency detectors can detect the infestation in grains up to 15 cm, the ultrasonic device (piezoelectric transducer) can detect infestation only when the infested seeds are in direct contact with the transducer (Hagstrum *et al*., 1988; Webb *et al*., 1985). In addition to detecting *C. maculatus* in cowpea, the ultrasonic detection system is applicable for other internal infesters such as *R. dominica* in rice, *S. oryzae* in maize, *S. cerealella* in wheat, and *Acanthoscelides obtectus* and *Zabrotes subfasciatus* in common bean (Shade *et al*., 1990). Betts (1991) used a crystal having piezoelectric properties that came in direct contact with a vibratory receiving structure for detecting vibrations due to insect feeding and locomotor activities inside grain. In a model study, Hagstrum *et al*. (1988) estimated the population density of *R. dominica* in 160 kg of wheat in a steel drum by acoustic detection and by a direct counting method. The acoustic detector system consisted of a piezoelectric sensor mounted on the end of a probe that was pushed into the grain, a battery-operated amplifier, and earphones. The results indicated that the acoustic method was comparable to that of the counting method. Subsequently, Hagstrum *et al*. (1991) used an automated acoustic system for monitoring *T. castaneum* population in stored wheat (135-kg lots) in steel drums. Four cables, each having piezoelectric microphones, had been positioned at 15-cm spaces vertically in the drum. It was noted that the number of insect sounds counted per 10-second interval was linearly related to insect density. They also observed that the number of insect sounds counted varied with time, distance between insect and microphones, and the developmental stage of the insect. In another study, Hagstrum *et al*. (1996) demonstrated automated monitoring using acoustic sensors for detecting infestation of *R. dominica, T. castaneum*, and *S. oryzae* in wheat stored on farms in bins in the United States. The detection limit was 0–17 insects/kg^{-1} of grain and the detection level was comparable to that of conventional sampling methods. However, 11.5% false positives and 15.2–40.0% false negatives in heavily infested bins and 52.2–85.7% false negatives in some of the lightly infested bins were observed. False positives have been attributed to electrical noise, whereas false negatives were due to failure to check the sensor more often.

Litzkow *et al*. (1990) also used a piezoelectric sensor to detect *S. oryzae, R. dominica*, and *S. cerealella* in cereal commodities including corn, wheat, and rice stored in containers such as trucks, ships, railroad cars, and storage bins. The piezoelectric sensor was placed in the container or attached to a probe for generating electricity in response to vibrations at frequencies above 500 Hz. Hickling *et al*. (1997a) considered that placing the hard, plane piezoelectric surface in direct contact with the commodity does not

provide an interfacial acoustic match comparable to the diaphragm or liquid-filled cushion in their invention; in addition, the piezoelectric element becomes less sensitive with pressure, such as would occur deep inside a grain silo. Use of piezoelectric sensors in acoustic systems enabled insect detection in larger grain samples also (Hagstrum *et al.*, 1988).

The intensity of the sound produced by insects is much lower when compared with that of ambient or background noise. For example, the larvae of *S. oryzae* in wheat produced sound to the level of 23 dB (20 μPa). Interference by background noise has been a challenge in the application of acoustic insect detection systems. In earlier studies, the detection was carried out in low-noise rooms or anechoic chambers. During acoustic detection of fourth instar larvae of *S. granarius* in wheat, the interference of ambient noise was eliminated by the use of 61 × 66 × 117 cm muffle-box made of 26-cm thick multilayered wood and foam (Mankin *et al.*, 1996). Vick *et al.* (1988a) constructed a small sound insulated room from wood, foam, and sound board that could reduce the background noise from 67 dB to 13 dB sound pressure level. Hagstrum and Flinn (1993) fabricated a 35 × 40 cm chamber from 1.9-cm thick plywood, foam, and sound barrier that could reduce laboratory noise sufficiently to allow detecting infestation in 1-kg grain samples. The problem of background noise, however, was overcome by placing infested grains directly on a sensor and measuring the ultrasonic signals produced.

Busnel and Andrieu developed a portable grain weevil detector in 1966 in France (Andrieu and Fleurat-Lessard, 1990). Vick *et al.* (1988b) showed that the sounds produced by feeding larvae of *R. dominica, S. oryzae*, and *S. cerealella* in grain samples could be detected by an attached acoustic coupler that consisted of an airtight cavity serving as the detector and a condenser microphone inserted into the other end to serve as the transducer. Hickling *et al.* (1997a) developed a detection system applicable for agricultural commodities such as cotton bolls, fruits, nuts, and grain. It consisted of (1) a structure that isolates the agricultural commodity from external noise and vibration, (2) an acoustic sensor with an electrostatic microphone having a −43 to −45 decibel sensitivity, and (3) user-recognizable outputs like earphones or a light-emitting diode. Electronic sound detection devices have been developed to enable automation in detection and monitoring infestation (Shuman *et al.*, 1993).

Sound produced by insects is transmitted principally through the intergranular air. In bulk storage, an important impediment in acoustic detection is the attenuation of insect-produced sounds by the grains (Hickling and Wei, 1995). Hickling *et al.* (1997b) conducted studies on the variation in sound transmission in different types of grain including hard and soft wheat, brown rice, soybean, corn, and sorghum. They observed that grain absorbs

insect-produced sounds and the attenuation coefficient increased as the square root of the frequency (kilohertz) of sound produced. The study revealed the need to install acoustic sensors at optimal levels for efficient detection and monitoring of infestation in silos and other bulk storage, because the acoustic sensors detect insects in grain at a maximum distance of 10–15 cm only (Hagstrum *et al*., 1991; Vick *et al*., 1988a). The levels of acoustic signals produced varied between species. Insects such as *R. dominica* and *S. oryzae* that cause more damage to grain, and *T. castaneum* with the highest locomotor activity, produced more acoustic signals and hence were more quickly detected than species such as *O. surinamensis* and C. *ferrugineus* that cause less grain damage (Hagstrum and Flinn, 1993). Although the sound spectra for *S. oryzae, R. dominica*, and *S. cerealella* larvae were similar on wheat, rice, and corn, the peak frequencies varied at 1200, 1475, and 587 Hz for wheat, rice, and corn, respectively. Similarly, the number of sounds detected for *S. oryzae, R. dominica*, and *S. cerealella* was more on rice, and it was lowest on wheat (Vick *et al*., 1988b). Insect-produced sounds are usually transitory signals covering a wide frequency band. Also, the sound produced by the feeding larvae (clicking sound) is clearly different from that of adults (feeding or movement). The acoustic signals from adults of *T. castaneum* were more than 80 times greater than their larvae (Hagstrum *et al*., 1991); the sounds produced by an adult *R. dominica* were more than 35 times that of their larvae at 27 °C (Hagstrum *et al*., 1990b). For larvae developing inside grains, the intensity of acoustic signals increased with advancing maturity (Shade *et al*., 1990; Vick *et al*., 1988b). Also, the feeding activity of larvae inside food grains is not continuous, and activity decreases when the grain is disturbed (or cooled). In a laboratory study, Mankin *et al*. (1999) concluded that grain samples should be left undisturbed for 15–20 minutes before acoustic detection/inspection. The influence of temperature on the levels of acoustic signals produced by insects varied depending on the species (Hagstrum and Flinn, 1993). For *S. oryzae*, the number of sounds increased from 17.5° to 35 °C and decreased afterwards; in *T. castaneum* the acoustic signals were lowest at less than 25 °C and increased subsequently; in *R. dominica* the signals increased from 17.5° to 30 °C, and thereafter the signals remained constant. The feeding activity of larvae of *Callosobruchus maculatus* in cowpea increased between 15° and 25 °C and then remained constant from 25° to 40 °C, and it decreased between 40° and 46 °C (Shade *et al*., 1990). Hickling *et al*. (1997a) noted that immersing the fruit or cotton bolls in warm water for a while could stimulate the larval activity and thereby increase the acoustic signal strength of the larvae. Mankin *et al*. (1999) also observed that insect activity and thereby detectability can be quickened by warming the sample so that the feeding activity of the larvae and locomotor activity of adults and the larvae are increased.

A good correlation between number of sounds and the number of insects detected has been established. The sounds of different insect species are, however, indistinguishable from one another (Vick *et al*., 1988b). A detection limit of five *S. granarius* or *S. oryzae* larvae or one adult per kilogram of grain has been reported by Fleurat-Lessard and Andrieu (1986). A still lower limit of detection of one mature larva of *S. oryzae* in 0.77 kg of wheat (Vick *et al*., 1988b) and two *T. castaneum* adults per 27.2 kg of wheat (Hagstrum, 1991) has been noted.

Very little attention has been paid to the acoustic detection of moth pests infesting stored food commodities. Male moths are known to produce ultrasonic sound to attract their females (Spangler, 1985). This ultrasonic sound is considered to play certain other roles such as detection of a food source and as a warning against predators (Spangler, 1988). Male *C. cephalonica* have been shown to produce trains of 125-kHz sound pulses similar to the echolocating sonar sounds of a typical bat (Spangler, 1987). Males of other pyralid moths such as *E. cautella, E. kuehniella*, and *Plodia interpunctella* also produce ultrasonic sounds up to 80 kHz by wing fanning during courtship behavior (Trematerra and Pavan, 1994). By detecting the ultrasonic sound produced by the male moths, it may be possible to locate *C. cephalonica* infestation in the vicinity. Such a detection method using a Polaroid electrostatic ultrasonic transducer has been used for lesser wax moth *Achroia grisella* in stored honeybee comb (Spangler, 1985). Infestation of stored product moths in flour mills, confectionery units, and similar food-processing facilities could be detected using suitable devices capable of detecting ultrasonic sounds produced by male moths.

The acoustic method is relatively rapid and particularly useful for the detection of active species like *T. castaneum, R. dominica*, and *S. oryzae*. The drawbacks of the acoustic technique include the following: (1) Sedentary stages such as eggs, pupae, and small larvae are not detected, (2) in bulk storage the sensors must be put at short distances (i.e., at every 15 cm), (3) insect activity (and therefore detectability) will vary with temperature, and (4) insect species present cannot be distinguished. Hagstrum (1991), however, believes that it is possible to discriminate among the species based on the number of voltage spikes (acoustic signals) from piezoelectric sensors due to insect-produced sounds.

V. CONCLUSION

There has been substantial progress in research in the area of insect detection for the past 65 years. Insect detection, in samples or in storage facilities, will continue to play a significant role as an effective management tool in the

food industry. The various methods developed have limitations of their own, limitations in their application, or limitations in interpretation of the results (Chambers, 2003). Sensitive ELISA-based methods have been developed to detect both single and multiple species in food commodities. Of late, PCR and direct fluorometry techniques have shown promise for pest detection. New systems involving imaging techniques and software-driven analysis have been introduced to known (x-ray) and newer (NIRS) detection methods (1) for automation, (2) to save people power, and (3) to save time. Nevertheless, we are yet to exploit technologies such as electronic nose and biosensor for infestation detection. We are still learning the complex chemical vibrations, and ultrasonic communication signals of insects to exploit them as a means of detection. There is a need to develop sensitive methods to detect the egg stages of insect pests (except *Sitophilus* spp.) in food commodities. Pheromone traps for all insect pests of stored foods are not yet commercially available (e.g., *Cryptolestes* spp.). Finally, there is a feeling among storage managers that modern insect detection and monitoring techniques are relatively expensive (when compared with control processes), so there is a need to minimize the cost of any detection technique. With some of the insect detection methods (e.g., acoustic and trapping methods), the correlation of the results to the actual infestation level in the sample, premises, or bulk storage is still not well defined.

ACKNOWLEDGMENTS

The author thanks the director, Central Food Technological Research Institute, Mysore for encouragement and Ms. Khamrunissa Begum for assistance in collecting references and typing the manuscript.

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