Detection of Resistance in Storage Pests: Sampling, Preparation, and Sending of Samples for Resistance Analysis

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CONTENT

Introduction: Why monitor resistance of storage pests?

- Resistance of pests means that these organisms become resistant to insecticides or other control methods that have traditionally been used to limit their damage. The problem of pest resistance is a global challenge that also concerns farmers and food producers in Europe.
- To address this issue, it is important to implement an Integrated Pest Management (IPM) approach, which includes a combination of different control strategies and targeted interventions. Targeted interventions against pests in commodity stores require knowledge of resistance. Sometimes, the effectiveness of insecticides and the spread of resistance are not adequately monitored, making it difficult to respond quickly to emerging issues. Most farmers, food producers, and commodity traders are unable to determine resistance on the spot. Therefore, it is necessary to collect samples (of pests or infested commodities).
- For this reason, a manual has been created that is intended for the targeted collection of samples in agricultural warehouses and facilities in order to obtain live storage pests for resistance testing.

Introduction - Multiple methods of sampling

- Targeted sampling in agricultural storage facilities and operations for the purpose of obtaining live stored pests can encompass several methods. An overview of the main techniques and approaches is included in the manual with a brief description or key terms. For non-specialized individuals, selected methods have been supplemented with illustrative photographs.
- The manual covers not only sampling from commodities but also from accumulated commodities and commodity residues from transport routes or near roadsides. This is because it has been scientifically proven (see literature below) that these areas can have a much higher density of pest infestations than the actual commodities.

Citations

Arthur, F.H.; Hagstrum, D.W.; Flinn, P.W.; Reed, C.R.; Phillips, T.W. (2006) Insect populations in grain residues associated with commercial Kansas grain elevators. J. Stored Prod. Res., 42, 226–239

Kucerova, Z., Aulicky, R., & Stejskal, V. (2003). Accumulation of pest-arthropods in grain residues found in an empty store/Akkumulation von Schadinsekten in Getreideresten in einem entleerten Speicher. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection, 499-504.

Morrison III, W. R., Bruce, A., Wilkins, R. V., Albin, C. E., & Arthur, F. H. (2019). Sanitation improves stored product insect pest management. Insects, 10(3), 77.

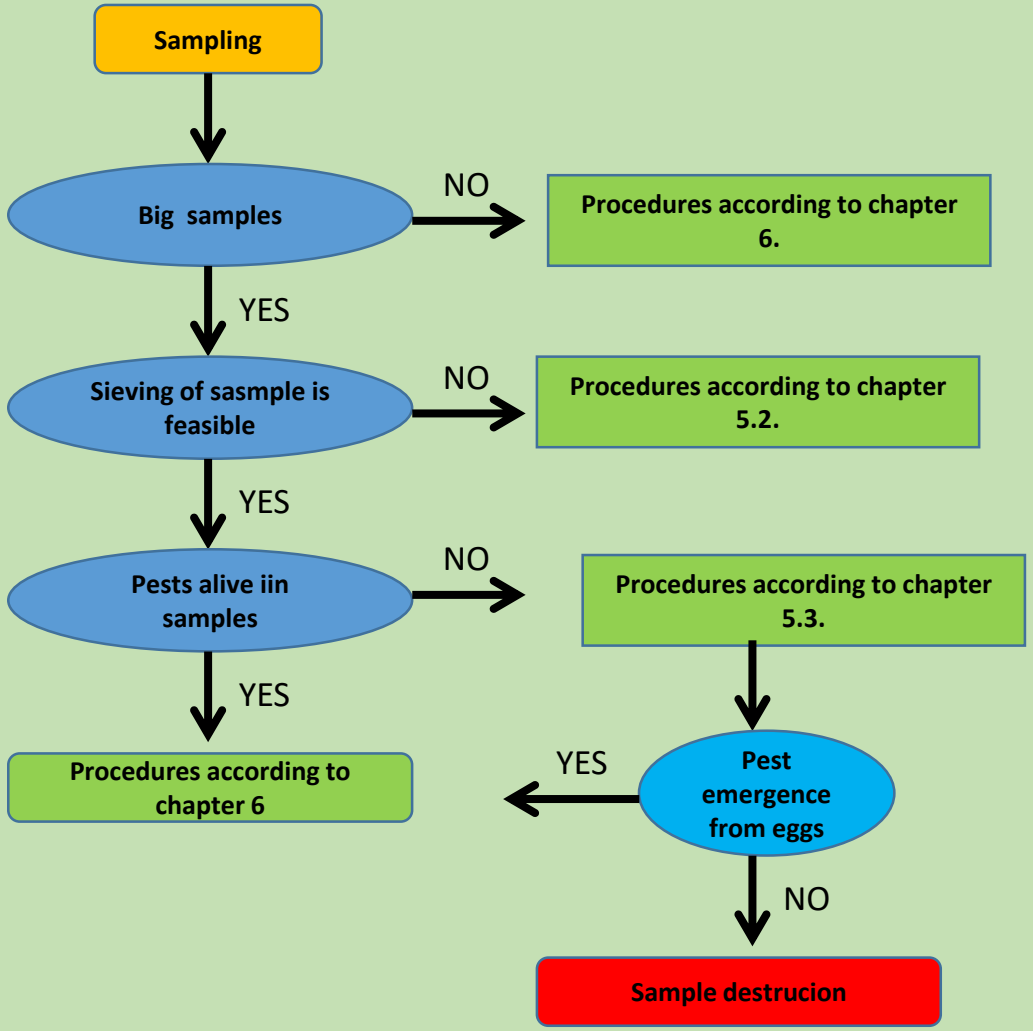
Tilley, D. R., Subramanyam, B., Casada, M. E., & Arthur, F. H. (2014). Stored-grain insect population commingling densities in wheat and corn from pilot-scale bucket elevator boots. Journal of Stored Products Research, 59, 1-8.

Tilley, D. R., Casada, M. E., Subramanyam, B., & Arthur, F. H. (2017). Temporal changes in stored-product insect populations associated with boot, pit, and load-out areas of grain elevators and feed mills. Journal of Stored Products Research, 73, 62-73.

Goals and limitations

- The manual is intended for the targeted collection of samples in agricultural warehouses and facilities in order to obtain live storage pests for resistance testing. It is applicabe for only adult individuals of target beetle species (Coleoptera). It does not cover other developmental stages of beetles and other insect orders.
- The manual is not intended for routine sampling of stored commodities for the purpose of detection, monitoring, or determination of insect pest population density, for example, according to standards such as EN ISO 24333:2009; EN ISO 24333:2009/AC:2010-08, etc.
- Sample collection with the intention of intentionally finding storage pests and direct collection of storage pests is considered to be the collection of genetic resources, and these samples must be handled in accordance with the Nagoya Protocol and REGULATION (EU) No. 511/2014 of the European Parliament and of the Council, as well as local legislative requirements of individual states.
- The mentioned sampling items and traps (photos, types) are provided as examples and do not imply a recommendation of a specific product brand.
- The determination of resistance is site-specific; although pests migrate within a facility, it cannot be ruled out that in an identical facility at a different location, in a different batch of grain, or in a different storage cell/silo, there will be a population with a different degree of resistance.

Sampling decision process



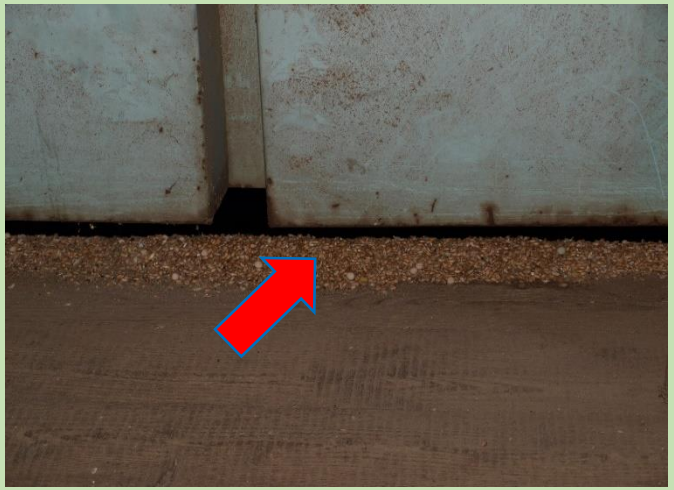
Sampling of pest and/or infested commodity

4.1

Residue Sampling in Warehouses

For the detection of pests within warehouses, it is optimal to sample residues of grain and other remnants, especially in hard-to-reach areas where sanitation might be compromised.

Sampling of grain (commodities) residues along the walls and in the corners of stores.



4.1

Sampling of residues in hard-to-reach areas of the warehouse:

4.1

Sampling of residues under pallets, around old or damaged bags:

4.1

Sampling of residues in cracks, crevices, or around entry doors:

4.1

Sampling of residues in technology, machinery, and transportation routes:

4.1

Sample the grain around pallets and old bags:

4.1

Sampling in hard-to-reach and neglected areas:

4.1

Sampling under doors, in cracks and crevices of walls and floors:

4.2

Sampling in transportation routes and service technologies

Grain storage facilities are equipped with transportation routes and technologies for cleaning, drying, or further processing of stored grain. The sanitation of these technologies is often neglected, or poor accessibility prevents regular inspection and cleaning. Consequently, these technologies accumulate residual remains, which serve as a food source and substrate for the further multiplication of a variety of storage pest species.

4.2 Machinery and technological equipment:

Cleaning machinery

Driers

Entry points

Active ventilation

4.3

Sampling of commodities upon receipt into the store

During grain intake, sampling is conducted to detect the presence of storage pests. This is one of the fundamental operational tasks aimed at uncovering any potential infestation of pests within the received commodity.

Depending on the form of the stored commodity during transportation, sampling is divided into sampling of loosely stored commodities and sampling of packaged commodities.

4.3.1. Sampling of loosely stored commodities on transport vehicles

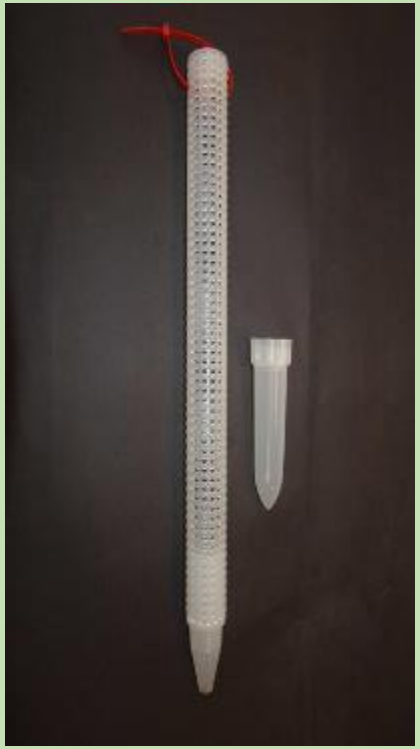
A pneumatic probe sampler (spear-type sampler with an automatic air-driven probe) is a modern device used for sampling grains, seeds, or other bulk materials from vehicles or other storage locations. The pneumatic probe sampler enables quick, automated, and representative sample collection, which is particularly important in the mass storage of bulk materials such as grains. This type of sampler allows for rapid inspection for the presence of pests.

The pneumatic probe sampler features an automated probe, which is a tube with a sharp end connected to a system that enables the probe to move up and down. The probe is inserted into the bulk material in the cargo space of a vehicle (truck bed) or another storage location. The precise position of the probe is controlled by an automated mechanism. Once the probe is correctly inserted into the material, an air system drives the probe's movement up and down. During this motion, samples of the material are collected within the probe. After obtaining the sample, the probe is retracted from the material. Subsequently, the air system draws the samples from the probe into a collection container, typically in an analytical laboratory setup.

4.4

Pitfall traps in commodities

- Surface traps (cone or cup traps)
- Subsurface probe-traps (probe traps)



4.5

Sampling of samples from commodities (bins, floor storage, big bags)

4.5 Sampling of samples from commodities (bins, floor storage, big bags)

Horizontal stores:

- Sampling from the subsurface layer using a scoop (measured at locations near walls and with higher humidity
- Sampling using double-walled spear (probe) samplers.

Sacks, bags, big bags

- Sampling of samples from the surface of open bags and sacks, or by probes if feasible."

- Spike samplers

Extraction of pests from large volumes of grain of samples

In the case of obtaining a large volume of samples that cannot be packaged and sent, it is necessary to first perform the extraction of pests from these samples.

5.1

Sifting and sieving of grain sample

Extraction of pests from grain samples is the most common method used in practice.

Sifting of samples can be performed using manual or automatic sieving with the help of sifters.

Sifting is carried out on screens with 2x2 mm openings, ensuring the passage of only developmental stages of pests in the intergranular space, along with dust particles and fragments. Whole grains and larger damaged grains remain on the sieve.

The material under the sieve is processed as a sample or only selected pest species are further extracted from it through manual collection

5.2

Manual collection of harmful beetles

If it is not possible to sieve samples for the purpose of pest extraction, manual collection of pests is necessary.

Samples obtained from storage facilities may contain a significant amount of dust particles or germinated and moldy grain, etc. Pests cannot be extracted from these samples through standard sieving methods.

For these reasons, manual collection of pests is employed. The sample is placed in a larger container and using a stereo microscope, a visual inspection of the sample is carried out, along with the collection of found pests.

5.3

Hidden grain infestation

The granary weevil (*Sitophilus granarius*), rice weevil (*Sitophilus oryzae*), and lesser grain borer (*Rhyzopertha dominica*) are classified as primary pests, as their developmental stages undergo their development inside grains. For this reason, extracting these developmental stages from samples through simple sieving is not feasible.

In cases where adults of these three pest species are not found in the samples, it does not mean that the sample is not infested by the developmental stages (eggs, larvae, pupae) of these pests. To verify infestation, samples can be stored under standard conditions, allowing the pests to complete their developmental cycle.

To determine hidden infestation, procedures according to the ISO 6639-3:1986 standard 'Cereals and pulses — Determination of hidden insect infestation — Part 3: Reference method' can be utilized

Development time (in days) for determining the developmental stages of insects in samples of cereals and pulses maintained under specified conditions according to the ISO 6639-3:1986 standard 'Cereals and pulses — Determination of hidden insect infestation — Part 3: Reference method'.

		Development time(days)	
Species		**25 °C**	**30 °C**
granary weevil	*Sitophilus granarius*	56	42
rice weevil	*Sitophilus oryzae*	56	42
lesser grain borer	*Rhyzopertha dominica*	70	49

Sample preparation and sample shipping

The goal of sample preparation and sample shipping is to ensure the safe transportation of selected species of storage beetles and their developmental stages to the laboratory for the purpose of assessing the resistance of these pests to selected active substances in plant protection products.

6.1

Sample preparation and storage

A properly prepared sample should ensure the safe transportation of adult individuals and their developmental stages.

Samples are prepared in secure and robust packaging to reduce the risk of package damage during transport or pest penetration.

Prepared samples are stored at optimal temperatures of 10 – 25°C.
Samples must not be exposed to direct sunlight.

Temporary sample storage: Place the samples into clean and sealed containers, which are appropriately labeled and recorded for further analysis.

Examples of sample (with pests + commodity) packaging

Solid and durable containers - optimal solution

Examples of sample packaging

Bags and pouches - less suitable types of packaging

Bags inside plastic containers :

6.2

Sending of samples

Packing of the sample:

Place the sample in the primary container into a secondary container (cardboard box). If necessary, secure the sample within the secondary container using padding to prevent movement during handling. Seal the secondary container and affix the recipient's address.

Address:
Name of the contact person 1)
Crop Research Institute
Drnovská 507/73
161 06 Praha 6 – Ruzyně
Czech Republic
No. Phone: 702 087 680

1) Vaclav Stejskal/ Radek Aulicky/ Tomas Vendl

7 Resistance evaluation

7.1 Active ingredients tested

Resistance is determined in selected species of storage beetles to three selected active substances used in products for treating stored commodities: phosphine, deltamethrin, and pirimiphos-methyl.

7.1.1. Phosphine

The evaluation is carried out using a modified standard international “Phosphine Tolerance Test Kit” (manufactured by Detia Degesch; Germany (DDPRK).

7.1.2. Deltamethrine

The evaluation is carried out by means of a modified FAO test, which is evaluate the discriminatory dose in a non-resistant laboratory strain CRI of each species studied for deltamethrin (preparation - K- Obiol 25 EC).
The resistance evaluation is possible in these species: granary weevil (*Sitophilus granarius);* rice weevil (S*itophilus oryzae) ;* lesser grain borer (*Rhyzopertha dominica)*; red flour beetle (*Tribolium castaneum);* confused flour beetle (Tribolium *confusum); s*aw-toothed grain beetle (*Oryzaephilus surinamensis).*

7.1.3. Pirimiphos-metyl

The evaluation is carried out by means of a modified FAO test, which is evaluate the discriminatory dose in a non-resistant laboratory strain CRI of each species studied for pirimiphos-methyl (preparation - Actellic 50 EC).
The resistance evaluation is possible in these species: granary weevil (*Sitophilus granarius);* rice weevil (S*itophilus oryzae) ;* lesser grain borer (*Rhyzopertha dominica)*; red flour beetle (*Tribolium castaneum);* confused flour beetle (Tribolium *confusum); s*aw-toothed grain beetle (*Oryzaephilus surinamensis) .*

7.2

The preparation of biological material for testing

To determine resistance to a single active substance, 200 adult individuals of each tested species are required.

If there is a shortage of individuals for testing, the laboratory will first breed them, and after obtaining a sufficient quantity, resistance tests will be conducted.

In the case of breeding pests to obtain a sufficient number of individuals for testing, there is a time delay of resistance/susceptibility verification by 3-6 months, depending on the number of captured individuals.

If the initial population consists of only a few individuals, the subsequent F1 generation has small numbers, and it is necessary to perform further breeding. Individuals of the F2 generation are subsequently used for testing.