SLOVENSKI STANDARD
SIST ISO 6639-3:1997

01-maj-1997

Žito in stročnice - Določanje prikritega napada insektov - 3. del: Referenčna metoda

Cereals and pulses -- Determination of hidden insect infestation -- Part 3: Reference method

Céréales et légumineuses -- Détermination de l'infestation cachée par les insectes -- Partie 3: Méthode de référence

Ta slovenski standard je istoveten z: **ISO 6639-3:1986**

ICS:

67.060	Žita, stročnice in proizvodi iz njih	Cereals, pulses and derived products

SIST ISO 6639-3:1997 **en**

International Standard 6639/3

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION●МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ●ORGANISATION INTERNATIONALE DE NORMALISATION

Cereals and pulses — Determination of hidden insect infestation —
Part 3: Reference method

Céréales et légumineuses — Détermination de l'infestation cachée par les insectes — Partie 3: Méthode de référence

First edition — 1986-12-15

UDC 633.1 : 635.65 : 632.7 **Ref. No. ISO 6639/3-1986 (E)**

Descriptors : agricultural products, cereal products, leguminous grains, tests, determination, insects, contamination, analysis methods.

Price based on 4 pages

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 6639/3 was prepared by Technical Committee ISO/TC 34, *Agricultural food products.*

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

Printed in Switzerland

INTERNATIONAL STANDARD ISO 6639/3-1986 (E)

Cereals and pulses — Determination of hidden insect infestation —
Part 3: Reference method

0 Introduction

This International Standard describes methods of determining hidden insect infestation in cereals and pulses. It consists of the following parts:

Part 1: General principles.

Part 2: Sampling.

Part 3: Reference method.

Part 4: Rapid methods.

1 Scope and field of application

This part of ISO 6639 specifies the reference method for determining the nature and number of hidden insects in a sample of cereals or pulses. Its aim is to count all the individuals, at every stage of life, of every insect species that normally feeds and develops within cereals and pulses.

The method is slow because it allows each insect to complete its developmental cycle and to emerge as an adult from the grain before it is removed. It can be used reliably for species that normally feed within grains, but not for species that occasionally feed in holes or cracks in grains. These may be shaken from the grains or be induced to leave them by the disturbance of handling at any stage of the life cycle and some are likely to be killed in the process. The numbers of such species will therefore be underestimated.

2 References

ISO 712, *Cereals and cereal products — Determination of moisture content (Routine reference method).*

ISO 5223, *Test sieves for cereals.*

3 Definitions

See ISO 6693/1.

4 Principle

Maintaining test samples at a controlled temperature and relative humidity such that the greatest possible proportion of the insects present in the sample when collected can develop to the adult stage. Removal of insects that emerge from the grains, identification and counting, at close intervals, to enable the number initially present to be identified.

5 Apparatus

Usual laboratory apparatus, and in particular:

5.1 Airtight containers, for storage of samples prior to the determination of moisture content (see ISO 712).

5.2 Balance, accurate to 1 g and capable of weighing at least 300 g.

5.3 Transparent containers, preferably made of glass or plastic, of a size capable of holding up to 300 g of the sample to be tested, in a layer having a depth not exceeding 50 mm.

5.4 Closures, to allow exchange of air, but to prevent insects and mites from entering or leaving the containers (5.3).

NOTE — Filter papers sealed in place with paraffin wax have been found to be suitable.

5.5 Sieves, of suitable aperture sizes to retain the grain but to allow individual insects to pass.

NOTE — For cereals, a sieve of aperture size 2 to 2,5 mm should be suitable, but for pulses a larger aperture size would be necessary to remove some Bruchid beetles. It is desirable for the sieve to have a deep bottom pan to collect the insects removed (see ISO 5223).

5.6 Shallow trays, preferably white enamel, of dimensions about 450 mm × 300 mm, with a rim about 10 to 20 mm deep, on which large samples can be spread, or **transparent Petri dishes,** of diameter about 200 mm, for smaller samples.

5.7 Flexible (entomological) forceps or **small brush** of soft hair about 10 mm long and not more than 2 mm in diameter, insecticide-free.

5.8 Room or **incubator**, capable of being maintained to within ± 1 °C of a temperature in the range 25 to 30 °C and at a relative humidity of either 60 to 65 %, or 65 to 70 %, as appropriate.

NOTE — It is essential that all apparatus and rooms used in connection with this method be kept free of insecticides or other chemicals harmful to insects.

6 Sampling

Use samples obtained as described in ISO 6639/2. The samples shall be protected from extremes of temperature and humidity and from exposure to direct sunlight, to reduce any changes in the insect population such as would result from killing by heat or cold or by dehydration.

7 Procedure

7.1 Determination of the moisture content of the laboratory sample

Determine the moisture content of a test portion taken directly from a laboratory sample intended for the determination of insect infestation, in accordance with ISO 712.

7.2 Test portion

Weigh the laboratory sample to the nearest 1 g and divide it into test samples, each weighing 200 to 300 g if the moisture content of the grain is less than 15 % (m/m) or 70 to 100 g if the moisture content of the grain is more than 15 % (m/m). Place each test sample in a container (5.3) with a suitable closure (5.4).

7.3 Determination

7.3.1 If insects are abundant and active, use the test sieve and pan (5.5) to extract them from the sample, taking care not to load the sieve more than three grains deep (if necessary, divide the sample for this purpose).

After sieving, or if the insects are not abundant and active, spread the grain in a single layer on a tray or dish (5.6) and remove all the insects found, using the flexible (entomological) forceps or small brush (5.7).

Identify all insects found in the test sample and record separately for each species the number of adults, and where possible pupae and larvae. If required, the numbers of living and dead insects shall be recorded separately.

After removing all insects return the test sample to its container (5.3).

Replace the closure (5.4) on the container and place the sample in the room or incubator (5.8).

If the moisture content as determined in accordance with 7.1 was above 15 % (m/m), ensure that the relative humidity of the room or incubator in which the sample is placed is between 60 and 65 %. If the moisture content is at or below 15 % (m/m) maintain the relative humidity between 65 and 70 %.

7.3.2 Repeat the procedures specified in 7.3 at regular intervals of 3 or 4 days for a period of at least 36 days. The actual length of the incubation period will depend upon the temperature at which the samples are stored, the type of grain involved and the species of insect present.

The recommended lengths of incubation periods for some insect species are given in the table. If more than one insect species is present in the sample, the incubation period for the species with the longest development shall be adopted.

Table — Incubation periods (in days) for the detection of the hidden stages of insects in cereal and pulses samples kept under the suggested conditions

Species	English common name	Incubation period (days)	
		at 25 °C	at 30 °C
Acanthoscelides obtectus (Say.)	Dried bean weevil	56	42
Araecerus fasciculatus Deg.	Coffee bean weevil	84	56
Callosobruchus maculatus (F.)	Cowpea beetle	49	35
Rhyzopertha dominica (F.)	Lesser grain borer	70	49
Sitophilus granarius (L.)	Grain weevil	56	42
Sitophilus oryzae (L.)	Rice weevil	56	42
Sitophilus zeamais Motsch.	Maize weevil	56	42
Sitotroga cerealella (Oliv.)	Angoumois grain moth	49	42
Zabrotes subfasciatus (Boh.)	Mexican bean weevil	56	42

8 Expression of results

NOTE — An example of a suitable data record sheet is given in the annex.

8.1 Record the numbers of insects found in each test portion at the first examination, by species and by stage (i.e. adults, pupae, larvae and eggs) and whether dead or alive, as required. Calculate the totals for all the test portions and, using the mass of the laboratory sample recorded in 7.2, express the initial observed infestation as a number per kilogram for each species and stage.

8.2 Record the numbers of insects found in all the test samples at each subsequent examination by species and stage and calculate the totals for all the test portions.

8.3 At the end of the final examination, calculate the totals for all the examinations and, using the mass of the laboratory sample recorded in 7.2, express the hidden infestation as a number per kilogram for each species and stage.

If any adult insects emerge from the test samples during the first 7 days of the examination period, adults of the same species emerging after the period recommended in the table shall be deducted from the total counted before the value for hidden infestation is calculated.

NOTE — It is assumed, in this case, that late emerging insects are progeny of adults emerging after the initial observed infestation has been removed, and, therefore, that they do not belong to the total infestation present at the time of sampling.

9 Interpretation of results

9.1 For each species, the pattern of emergence represents the age distribution at the time the sample was taken. The pattern, when plotted on a graph from right to left, will present a picture of the proportions of life stages from egg to adult in equal time periods.

A high proportion of young stages (late emergents) is a sign that the population in the zone sampled is increasing while a low proportion is a sign that the population is decreasing.

9.2 The significance of the number of insects found depends upon the temperature at which the product is stored. At temperatures below 15 °C, none of the species listed in the table can multiply quickly enough for small populations to be dangerous, but at temperatures above 25 °C, the presence of even a single individual per kilogram of any of the listed species is a serious hazard.

10 Test report

The test report shall show the method used and the results obtained. It shall also mention all operating details not specified in this part of ISO 6639, or regarded as optional, together with details of any incidents likely to have influenced the results.

The test report shall include all the information necessary for the complete identification of the sample.

Annex

Suitable data record sheet

Laboratory sample No. . . . . Mass . . . . g Moisture content . . . . % (*m*/*m*)

Insect species (stage)	Test portion	Number found													Totals	
		First examination	Incubation period (days)												in sample	per kilogram
	1															
	2															
	3															
	4															
	Total															
	1															
	2															
	3															
	4															
	Total															
	1															
	2															
	3															
	4															
	Total															
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	Total															