Seed Health:










Rules and Regulations for the Safe Movement of Germplasm

Second edition

M. Mezzalama

CIMMYT

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International Maize and Wheat Improvement Center

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Seed health procedures for incoming seed

These procedures are summarized in Figure 1.

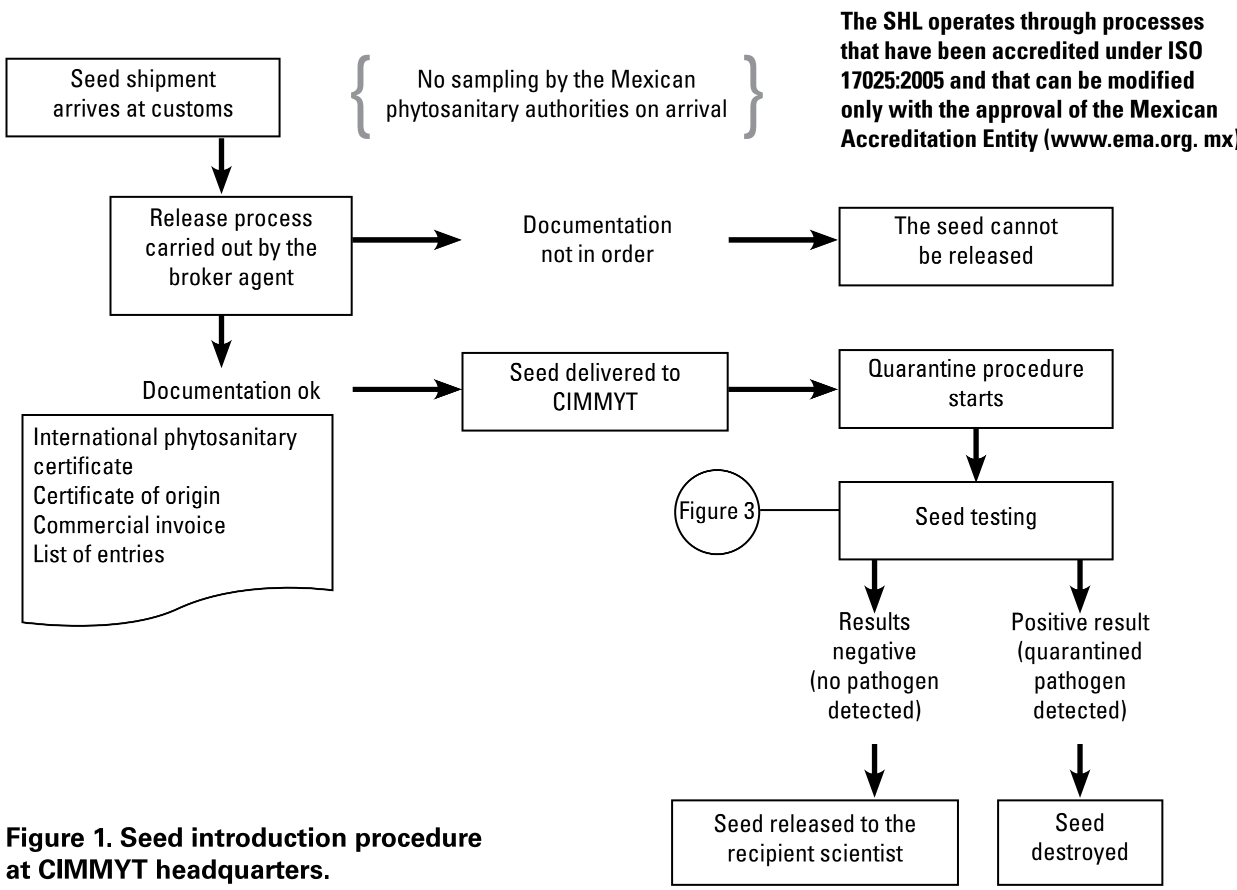

Figure 1. Seed introduction procedure at CIMMYT headquarters.

Required documents

Since 25 March 2004 CIMMYT has been recognized by the Mexican Government as an international center with international status. Despite this recognition, CIMMYT is not exempt from abiding by Mexican phytosanitary laws regarding the import of germplasm into the country.

Mexican authorities require that a permit be issued to any party (private or public company, international organization, etc.) intending to import seed or vegetative material into Mexico, for any purpose.

Mexico's plant health authority, Dirección General de Sanidad Vegetal (DGSV), stated on 4 December 1997 that CIMMYT must request an import permit prior to receipt in Mexico of any maize, wheat, or triticale seed. The import permit application must be accompanied by bibliographic references documenting all seed-borne and seed-transmitted pathogens present in the seed's country of origin.

Ordinary permit for crop seed entering Mexico

The ordinary seed import permit issued by Mexico is more strictly known as a "sheet of phytosanitary requirements" (*Hoja de Requisitos Fitosanitarios*); this is effectively an import permit but issued for seed being imported for non-profit purposes. It normally takes 2-3 weeks to be granted, and remains valid for six months after issue. Once the import permit is granted, CIMMYT will send a copy to the exporting collaborator. Instructions outlining proper import procedures **must be followed strictly** to ensure successful importation. If any required original document is missing, the entire shipment will be destroyed without recourse or reimbursement.

The following documents must accompany seed destined for importation to Mexico.

- A copy of the seed import permit granted by the Mexican Government specifically for the country of origin. The original is kept at CIMMYT headquarters;
- The original letter of commercial value (Appendix 3);
- The original certificate of origin (Appendix 4);
- The original international phytosanitary certificate issued by authorities in the country of origin, including a description of the fungicide used to treat the seed.

Copies of these documents must be included in the box(es) containing the seed. The **originals** should be sent under separate cover but at the same time as the seed shipment. The originals may alternatively be included with the seed, but should be securely placed inside the box to avoid their being lost when the box is inspected at customs. If the original import documents are lost or misplaced before arriving in Mexico, the Mexican authorities will not release the shipment.

Those intending to send seed to CIMMYT should contact their CIMMYT collaborator before doing so for details of sending procedure.

Special permission for importing vegetative material into Mexico

A special import permit is required in the following cases:

- Seed not supported by international phytosanitary certificate (gene bank "black boxes")
- Vegetative tissue (leaves, bulbs, etc.)
- Fungal and bacterial cultures or isolates
- DNA extracts
- Plasmids

Permit applications for importing the above types of materials must be fully and carefully justified. The application must include the material's precise place and country of origin, its description (variety, weight, and any other useful information), and the aim or purpose of the special importation with a brief description of the research objectives and destination within the country.

Special import permits require three weeks of processing time, provided the information submitted is fully satisfactory to the granting authorities. If not, CIMMYT will be asked to provide additional information, and the application process will be restarted. This type of permit is valid ONLY for the specified material. If a shipment is different in content or quantity (even by only one gram of seed or one leaf) from that specified on the permit, it will not be released.

Seed health testing

Role of CIMMYT's Seed Health Laboratory (SHL)

The DGSV authorities officially recognize several private and public laboratories in Mexico where seed health testing may be conducted. All imported seed must be checked by one of these laboratories before release, at considerable expense.

Since 1988 the CIMMYT SHL has been officially authorized by DGSV to carry out the quarantine procedures on seed introductions coming into Mexico and CIMMYT, and in April 2007 the

SHL obtained accreditation under standard ISO/IEC 17025: 2005, "*General requirements for testing and calibration laboratories,*" as required by the Mexican government. An official DGSV inspector is assigned exclusively to CIMMYT to assist with thorough and timely seed inspection and importation.

The SHL's official status facilitates seed importation into Mexico (and CIMMYT) for experimental purposes, as follows:

- It avoids seed sampling and inspection at the point of entry (e.g., Mexico City airport), since it is done at CIMMYT by the internal inspector. This ensures that boxes enter CIMMYT as originally packed and unopened.
- Seed inspection and clearance are expedited.
- It ensures that phytosanitary inspection procedures are meticulously followed.

Laboratory testing

All seed brought into CIMMYT, without exception, **must be subjected to quarantine procedures in the Seed Health Laboratory.**

The inspector will check phytosanitary documentation, open the box, and macroscopically inspect the seed for smut sori, nematode galls, ergot sclerotia, weed seeds, insect damage, etc. Based on this inspection and depending on the amount of seed in the shipment, a standardized sampling procedure is applied to obtain the sample to be used for laboratory testing. For details on testing see the section on "Seed health testing procedures" (p. 10).

Consignees are informed that the SHL has received the seed with an e-mail report that includes a description of the shipment (arrival date, consignee, origin, list of entries, number of entries, weight, purpose, expected date of release, SHL registration number). These data and results of seed testing are also entered in a file on CIMMYT's Intranet page.

Wheat pathogens considered of quarantine interest in Mexico are listed in the standard *Norma Oficial Mexicana* NOM-017-FITO-1995, and maize pathogens in the *Norma Oficial Mexicana* NOM-018-FITO-1995, established by Mexico's Ministry of Agriculture, Livestock, Rural Development, Fisheries, and Food (SAGARPA). CIMMYT also has the responsibility to prevent the importation of any other organism considered potentially harmful to these crops by SHL staff (Tables 3 and 4, Appendix 2).

The SHL also ensures that shipments are free of weed species, regulated by *Norma Oficial Mexicana* NOM-043-FITO-1999, and from Khapra beetle (*Trogoderma granarium*), regulated by *Norma Oficial Mexicana* NOM-005-FITO-1995.

Results

The official inspector and the SHL staff judge whether or not a shipment should be approved for release on the basis of test results. It will be **released** if:

- The seed is pathogen-free, or
- The seed is free of pathogens of quarantine concern in Mexico but contains other undesirable pathogens (Tables 3 and 4, Appendix 2). In this case, the SHL will recommend appropriate treatment, to be applied before planting.

In both cases the SHL sends a notice of release to the consignee.

The inspector will **reject** the seed if:

- The seed carries any pathogen quarantined by Mexico's plant health authorities. In this case the seed will be handled according to the Mexican phytosanitary authorities' instructions. For example, it may be destroyed through incineration or treated.

Every month the SHL must send DSGV authorities a report of all seed introductions arriving at CIMMYT and an update on introduction releases.

Introduction blocks

Seed meeting the requirements described above may be released and picked up by a program-designated assistant only and planted without exception in the introduction blocks. These introduction blocks, or quarantine plots, are dedicated plots on CIMMYT's Mexican research stations set aside for the planting of newly-introduced maize or wheat seed. The purpose of this procedure is to monitor, detect, and destroy any pathogen that may have not been detected by laboratory tests.

All introduction blocks are grown in complete isolation, and intercropping of other materials is not allowed. Introduced seed will be monitored during the growth cycle by SHL staff and the DGSV inspector.

Each program will provide lists of all materials to be grown in quarantine plots before each cycle and in every location.

To facilitate inspection, materials being grown on introduction blocks should be labeled as soon as possible. The SHL introduction number, number of entries, and country of origin must be included on each tag.

All decisions regarding chemical spraying will be taken jointly by program scientists and SHL staff. Plants in the introduction blocks that show symptoms of unusual diseases will be removed, samples will be taken to the SHL for examination, and the remaining tissue will be destroyed.

Seed health procedures for outgoing seed

These procedures are summarized in Figure 2.

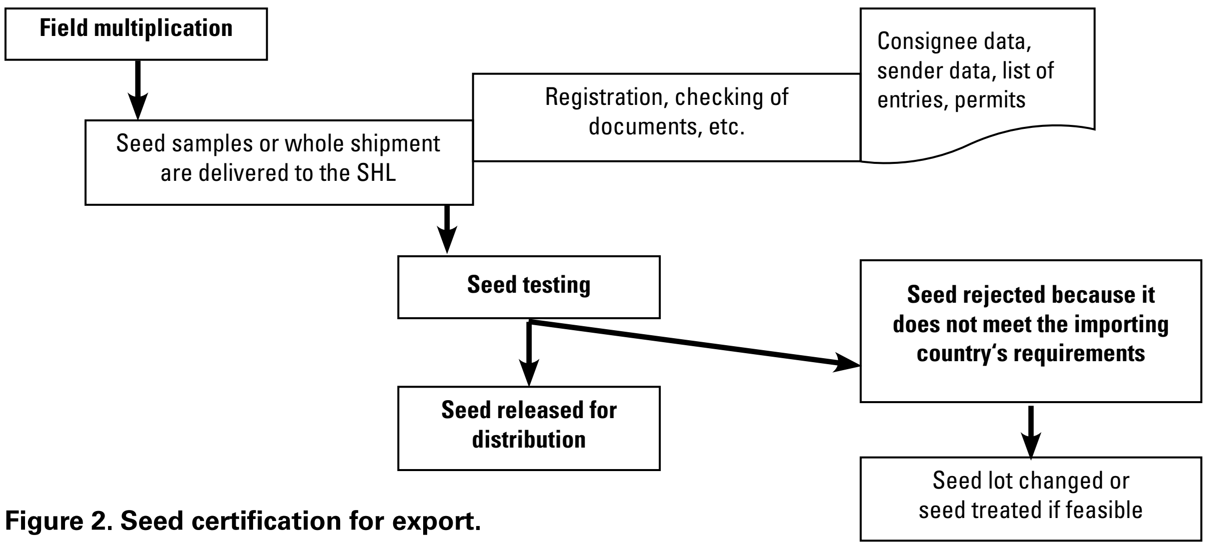

Figure 2. Seed certification for export.

Required documents

All maize, wheat, and triticale germplasm exported by CIMMYT either in an international nursery or a miscellaneous shipment must be accompanied by the following documents:

- An international phytosanitary certificate issued by the Mexican phytosanitary authorities
- A CIMMYT seed health certificate (in either English or Spanish) issued by the SHL
- Declaration of value
- A GMO-free declaration when required by importing country

Certification by CIMMYT is based on examination of samples of all seed and the use of tests conducted by the SHL including seed washing and filtration, greenhouse tests, incubation, and serological tests.

CIMMYT maize, wheat, and triticale seed is dispatched as clean as possible, to avoid the spread of seed-borne plant diseases and rejection of the seed shipment on arrival in the country of destination. When countries with very strict import regulations require an untreated seed sample, this is sent separately for testing on arrival.

Quarantine regulations all over the world categorize regulated pests A1 or A2. A1 pests are quarantine pests not present in a given area and A2 pests are quarantine pests present in that area but not widely distributed and thus under official control. All CIMMYT maize, wheat, and triticale seed that is shipped must be free from A1 and A2 pests.

For details on the laboratory tests carried out by the SHL to detect possible seed-borne and seed-transmitted pathogens see the section on "Seed health testing procedures" (p. 10). Additional tests are conducted upon request if the country of destination has special requirements.

Wheat

Seed multiplication in Mexicali, Baja California, Mexico (Karnal bunt-free area)

The Mexicali area, in northwestern Mexico has been officially declared free of Karnal bunt (KB) according to the *Diario Oficial de la Federación* published on 15 December 1997, following instructions given in *Norma Oficial Mexicana* NOM-001-FITO-2001. The area is also recognized as KB-free by the North American Plant Protection Organization (NAPPO) and the European Plant Protection Organization (EPPO).

The CIMMYT Wheat Program uses fields in this certified zone (Mexicali) for multiplying seed intended for export.

Seed planting procedures in Mexicali

Wheat and triticale germplasm to be included in an international nursery must be planted in plots assigned for that purpose at CIMMYT's headquarters at El Batán, State of Mexico, an area free of *Tilletia indica*, the causal agent of Karnal bunt.

During the crop cycle, plots are sprayed with the systemic fungicide propiconazole every 10 days from spike emergence to the end of grain filling, to protect the plots from aerial infection by *T. indica*.

After harvest, the seed is washed with a 1.2% sodium hypochlorite solution to destroy teliospores on the seed surface. Seed from each line is sampled by the SHL, and filters from seed washings are examined under a stereo microscope (Appendix 1). If no contamination is detected, the seed is treated with a mixture of carboxin + captan (Vitavax 300, 3 g/kg of seed) and chlorothalonil (Daconil 2787, 2 g/kg of seed). The seed is then shipped to Mexicali to be sown and multiplied.

During the Mexicali crop cycle, the germplasm is constantly monitored to produce seed of the highest quality. It is sprayed 2-3 times with propiconazole following the method described above. All sowing and harvesting machinery in Mexicali remains there, i.e., it is never moved to non-certified areas where the disease might be present. Visitors to CIMMYT multiplication plots in Mexicali are required to bring clean clothes and shoes to wear and to wash their vehicles before arrival.

The crop is inspected at the end of the cycle, close to harvest time. Randomly sampled spikes are collected, individually threshed, and examined to detect potential seed health problems. The seed then undergoes a filter wash test (Appendix 1). If the test results are negative, each line is individually harvested.

The harvested seed is placed in new bags that have been appropriately tagged. The new bags and tags to be used at harvest must be shipped by air to Mexicali. Harvested seed is appropriately packed and transported to El Batán in a sealed truck that does not pass through KB-contaminated areas.

To avoid contamination, the truck is vacuumed and washed before being loaded. Before entering the CIMMYT station at El Batán, the truck exterior is washed and vacuumed once again, to get rid of any contaminating pathogens, soil, or debris. The truck is then parked in a restricted area reserved exclusively for international nursery seed.

The inside of the storage area at El Batán is washed with a 1.2% sodium hypochlorite solution, and a sample of the wash water is taken for analysis to confirm that the area is free of any contaminants. There is zero KB tolerance in all areas and during all procedures conducted to ensure non-contamination.

Lines received in the international nurseries area can now undergo final selection by the program head for inclusion in international trials. The selected lines are cleaned by sifting through screens to eliminate weed seed and large debris. Each line is sampled and tested for the presence of viruses, bacteria, and fungi. Before international distribution, every line of every nursery is also washed using the following procedure:

The total weight of a line (if exceeding 1 kg) is divided into 800–1000 g portions and placed in perforated metal trays. Trays containing seed are then:

1. rinsed for 3 minutes with water + 10 ml of Tween 20 at high pressure (50-60 pounds/inch^2);
2. immersed for 3 minutes in 1.2% sodium hypochlorite solution; and
3. rinsed for 2 minutes with water at high pressure.

After washing, a further seed sample (of about 50 g) is taken from each tray and again sent to the SHL. Filter tests for the presence of *T. indica* and other *Tilletia* spp. teliospores are carried out as described in Appendix 1.

While the tests are being conducted, the remaining seed, after washing, is treated with a slurry containing chlorothalonil (Daconil 2787, 2 g/kg of seed) and carboxin + captan (Vitavax 300, 3 g/kg of seed), plus an adhesive. The seed is oven dried at 30°C until 9% moisture content is reached. If test results are positive for the presence of *T. indica* teliospores, or any other pathogen known to be quarantined, the line is discarded, even if it has already been treated.

Once it is washed, treated, and dried, seed is placed in envelopes, and the nursery is assembled for shipping to CIMMYT partners. Seed selected by visitors during the Yaqui Valley, Sonora cycle (see "Miscellaneous shipments" below) must follow this same procedure.

Miscellaneous shipments

"Miscellaneous" or "special" shipments contain seed that has not been through a multiplication cycle in Mexicali, but which is required to be sent to collaborators. CIMMYT's El Batán research station was declared free from Karnal bunt by the Mexican Ministry of Agriculture on October 20, 2009 (see *Diario Oficial de la Federación*, http://dof.gob.mx/index.php?year=2009&month=10&day=20). However, seed not multiplied in Mexicali merits special care because seed produced in El Batán or Toluca (CIMMYT's highland research station) is of lower quality, mainly due to environmental conditions (high rainfall) and higher disease incidence, and because the crop is not always sprayed with fungicide during the cycle as it is in Mexicali.

Every line is visually inspected to determine the seed's overall condition. When seed quality is very low (5-10% of the seed shows spots or other malformations), the breeder is strongly urged to select the seed for quality before delivering it to the SHL and shipping it to collaborators.

Accurate laboratory examination is required for Karnal bunt and other diseases of quarantine concern. SHL staff decide whether to test every line or make up a composite sample for testing. Seed must be washed and chemically treated (as described above) before shipment.

Seed produced on CIMMYT's Yaqui Valley, Sonora research station must go through a multiplication cycle in El Batán before export or multiplication in Mexicali.

Maize

Most maize seed destined for international distribution is produced on CIMMYT's maize research stations in Tlaltizapán (State of Morelos) and Agua Fria (State of Puebla). During the crop cycle, nurseries are periodically inspected in the field, and plants showing symptoms are discarded. After harvest, all seed is carefully checked for seed-transmitted pathogens of quarantine interest in countries to which nurseries will be sent.

The germplasm submitted by the Maize Program to the SHL falls into three groups: international nurseries, miscellaneous nurseries, and CIMMYT maize lines. Tests carried out by the SHL on maize seed are discussed in the section on "Seed health testing procedures" (p. 10). Maize seed is treated before shipment with a slurry containing thiodicarb (an oxime carbamate insecticide), captan, metalaxyl-M, thiabendazole (fungicides), and an adherent.

Rules for storing wheat seed under conditions of zero tolerance for Karnal bunt teliospores

Preventive seed health procedures to be applied before placing wheat seed in storage

Warehouse hygiene

Warehouses must be washed and cleaned regularly. Floors and surfaces must be disinfected with a 1.2% sodium hypochlorite solution. To check for contamination, slide traps must be positioned in every chamber, room, and common area and renewed periodically.

Seed testing before storage

Seed to be placed in storage should have been multiplied in areas free of Karnal bunt (KB) and subjected to SHL procedures as follows:

- Seed is tested for viruses, bacteria, and fungi by the SHL.
- To avoid reducing seed viability, seed should not be washed or treated with fungicide. Furthermore, for health reasons, CIMMYT staff should not work with fungicide-treated seed unless absolutely necessary.
- Seed is rechecked for KB before shipping to collaborators.

Seed is stored in metal containers to avoid contamination. Upon storage, SHL staff will set up slide traps to detect any contamination.

Preventive measures to be followed by warehouse staff

The number of staff supervising incoming and outgoing materials must be kept to a minimum.

When moving seed or entering the storeroom, staff must wear clothing (aprons or overalls) and footwear reserved specifically for this purpose. Laboratory coats must be washed regularly (every week) even if they have not been worn.

Procedures for cleaning the warehouse after seed is introduced

The storage area must be vacuumed regularly (at least once a week) to get rid of dust. It should never be dusted with a dry cloth. Dust collected in the vacuum bag must be placed very carefully into another bag and burned in an incinerator.

A cloth moistened in a 1.2% sodium hypochlorite solution may be used to wipe work surfaces. However, it should not be used on metal surfaces that have not been protected from chlorine by anti-corrosion paint (of the kind used in swimming pools).

Rules for moving wheat seed within Mexico from Karnal bunt-contaminated to Karnal bunt-free areas

Mexico's DSGV regulates wheat seed movement within Mexico from areas where KB is present to areas free from the disease, according to *Norma Oficial Mexicana* NOM-001-FITO-2001. Therefore:

- All seed shipments must be accompanied by a seed health certificate authorizing seed movement within Mexico (*Certificado fitosanitario para la movilización nacional*).
- All seed must be treated with chlorothalonil (Daconil 2787, 2 g/kg of seed).
- All seed produced in the CIANO-CIMMYT research station in the Yaqui Valley, Sonora, and arriving at the El Batán or Toluca research stations, must be inspected on arrival by a technician accredited by DSGV.

It should be stressed that seed from the Yaqui Valley may be sown only at El Batán and Toluca.

Seed health testing procedures

All maize, wheat, and triticale seed, both entering and leaving CIMMYT, must pass through the seed health laboratory. All seed undergoes the same set of testing procedures, although the key target pathogens vary between incoming and outgoing seed depending on relevant quarantine regulations.

CIMMYT generally uses well-established test procedures that may be found in any standard reference on seed health (see References). The filter wash test is somewhat specialized, and the procedure may be found in the appendix. The tests used in CIMMYT are as follows:

- **physical inspection** for smut sori, nematode galls, ergot sclerotia, weed seeds, insect damage, etc.

- **seed wash filter test**, which reveals the presence of fungal spores—including bunt teliospores (*Tilletia* spp.), smut spores (*Urocystis* and *Ustilago* spp.), and downy mildew oospores (*Peronosclerospora* and *Sclerophthora* spp.)—and of nematode cysts. This test takes around three hours, although large volumes of samples may take longer. Composite samples of outgoing seed may be used (with rechecking of individual lines in the event of a positive result).

- **freezing blotter test**, which reveals the presence of imperfect fungi carried by seed and takes two weeks.

- **greenhouse germination test**, for the expression, and thus detection, of seed-borne pathogens, and to check seed viability. This test takes three weeks. If symptoms appear on seedlings, further testing to identify the causal pathogen is carried out (i.e. ELISA or other tests).

- **ELISA**, or enzyme-linked immunosorbent assay, to detect specific bacteria and viruses. This test takes 24 hours.

- **immunofluoresence test**, an immunological assay, taking six hours, for the detection on wheat seed of *Xanthomonas translucens* pv. *undulosa*.

- **downy mildew detection test,** with microscopic examination, to detect *Peronosclerospora* and *Sclerophthora* spp on maize. This test takes 24 hours.

Details of the tests and their target pathogens are summarized in Tables 1 (maize) and 2 (wheat and triticale); a flowchart of the test procedures is given in Figure 3.

Table 1. Seed health testing on maize.

Test	Pathogen type(s) detected	Pathogens of importance in incoming seed	Pathogens of quarantine importance in outgoing seed **
Seed wash filter test	Fungi (smuts)		*Ustilago maydis*
	Nematodes	*Heterodera zeae**	
Freezing blotter test	Imperfect fungi	*Acremonium maydis**	*Cochliobolus* spp. *Dilpodia* spp. *Fusarium* spp. *Lasiodiplodia theobromae*
Greenhouse germination test	Bacteria	*Burkholderia andropogonis** *Clavibacter michiganensis* subsp. *nebraskensis** *Pantoea stewartii**	*Acidovorax aveanae* subsp. *avenae* *C. michiganensis* subsp. *nebraskensis* *P. stewartii*
	Viruses	Wheat streak mosaic virus*	Maize dwarf mosaic virus Maize chlorotic dwarf virus Sugarcane mosaic virus
ELISA	Bacteria	*Pantoea stewartii**	*P. stewartii*
	Viruses	Wheat streak mosaic virus*	Maize dwarf mosaic virus Maize chlorotic dwarf virus Sugarcane mosaic virus
Downy mildew detection test		*Peronosclerospora maydis** *P. philippinensis** *P. sacchari** *P. sorghi* *Sclerophthora rayssiae* var. *zeae**	*P. sorghi*

* Quarantined under *Norma Oficial Mexicana* NOM-018-FITO-1995.
** According to information reported on importing countries requirements

Table 2. Seed health testing on wheat and triticale.

Test	Pathogen type(s) detected	Pathogens of importance in incoming seed	Pathogens of quarantine importance in outgoing seed **
Seed wash filter test	Fungi:		
	bunts	*Tilletia indica**, *Tilletia controversa**	*Tilletia indica* *Tilletia* spp
	smuts		*Ustilago* spp
	Nematodes	*Anguina tritici**	.
Freezing blotter test	Imperfect fungi	*Alternaria triticina**	*Fusarium* spp. *Helminthosporium* spp. *Septoria* spp.
Greenhouse germination test	Bacteria	*Pseudomonas syringae* pv. *atrofaciens** *Xanthomonas translucens* pv. *undulosa*	*P. syringae*
	Viruses	Barley stripe mosaic virus Wheat streak mosaic virus	
ELISA	Viruses	Barley stripe mosaic virus Wheat streak mosaic virus	Barley stripe mosaic virus Wheat streak mosaic virus
Immunofluorescence		*Xanthomonas translucens* pv. *undulosa*	

* Quarantined under *Norma Oficial Mexicana* NOM-017-FITO-1995.
** According to information reported on importing countries requirements

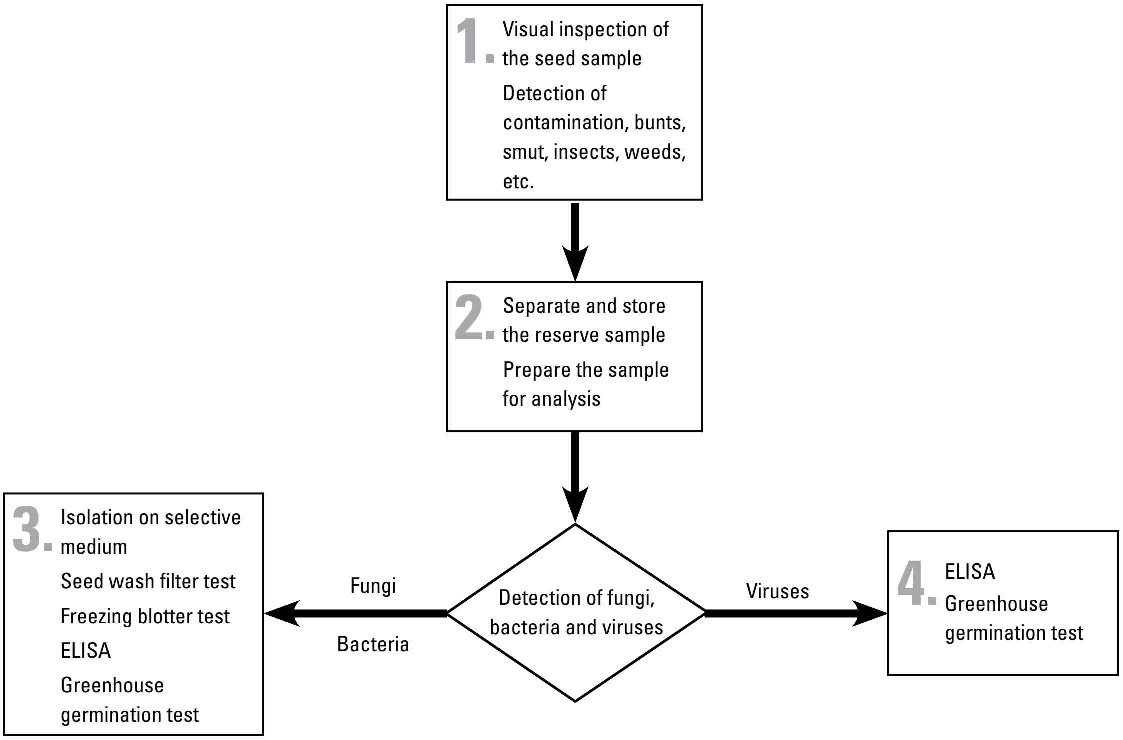

Figure 3. Pathogen detection and identification flowchart.

References

CAB International. 2007. *Crop Protection Compendium, 2007 Edition*. Wallingford, UK: CAB International.

ISTA. 2002. *Handbook on Seed Health Testing*. Section 2: Working Sheets. Edited by J. Jorgensen. Zürich: International Seed Testing Association.

ISTA. 2004. *Seed Sampling*. 2nd Edition. Zürich: International Seed Testing Association.

ISTA. 2009. *International Rules for Seed Testing*. Zürich: International Seed Testing Association.

Mathur, S.B, and B.M Cunfer (eds.). 1993. *Seed-borne Diseases and Seed Health Testing of Wheat*. Copenhagen: Danish Government Institute of Seed Pathology for Developing Countries. 168 pp.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists*. St. Paul, MN: APS Press. 150 pp.

Warham, E.J., L.D. Butler, and B.C. Sutton. 1996. *Seed Testing of Maize and Wheat: A Laboratory Guide*. Mexico, D.F.: CIMMYT. 84 pp.

Appendices

Appendix 1: Procedure for seed wash filter test

This test is for detecting the presence of spores of bunts and smuts, conidia of imperfect fungi, and oospores, either as contaminants or on asymptomatic seed.

Place a 10 or 20 g seed sample in 100 ml of water plus 2-5 drops of Tween 20 and agitate on a shaker for 30 min at 250-300 rpm (if Tween is not available, 1 drop (= 10 μl) of neutral liquid laundry detergent can be used). Filter the wash water through 53 and 15 μm polyester or nylon meshes. Large spores and debris will remain on the 50 μm mesh, and *Tilletia indica* teliospores, with an average diameter of 25-40 μm, will be caught on the 15 μm mesh. Add a few drops ok 3% KOH solution to the mesh and examine under a stereo microscope for the presence of spores. Mark any suspicious structures and confirm their identity under a compound microscope.

Instead of a 15 μm mesh a Whatman #1 filter paper can be used, placed in a Buchner funnel that is inserted into a flask attached to a vacuum pump. The Whatman #1 filter paper will trap all structures smaller than 50 μm. Similarly, after filtering, it should be moistened with 3% KOH and observed under a stereo microscope.

Appendix 2: Lists of seed-transmitted pathogens

Table 3. Maize pathogens transmitted by seed, with transmission levels and sources.

VIRUSES	
Maize chlorotic mottle virus	Transmitted at a low level. Delgadillo-Sanchez, F., J.L. Pons-Hernandez, and A.D. Torreon-Ibarra. 1994. Seed transmission of maize chlorotic mottle virus. *Revista Mexicana de Fitopatologia* 12: 7-10. In: CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International.
Maize dwarf mosaic virus	Transmission at 0.5-2.5%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp. Shepherd, R.J., and Q.L. Holdeman. 1965. Seed transmission of the Johnsongrass strain of the sugarcane mosaic virus of corn. *Plant Disease Reporter* 49: 468-469.
Sugarcane mosaic virus	Transmitted at a very low level. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. Mikel, M.A., C.J. D'Arcy, and R.E. Ford. 1984. Seed transmission of maize dwarf mosaic virus in sweet corn. *Phytopathologische Zeitschrift* 110(3): 185-191.
Wheat streak mosaic virus*	Transmission at 0.1%. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
BACTERIA	
Acidovorax avenae subsp. *avenae*	Transmission at 2–4%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. Dange, S.R.S., M.M. Payak, and B.L. Renfro. 1978. Seed transmission of *Pseudomonas rubrilineans*, the incitant of bacterial leaf stripe of maize. *Indian Phytopathology* 31(4): 523-524. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
*Burkholderia andropogonis**	Not proven to be seed transmitted. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. Elliott, C., and E.F. Smith. 1929. A bacterial stripe disease of sorghum. *Journal of Agricultural Research* 38: 1-22. Hernandez, Y., and G. Trujillo. 2001. Detection of phytopathogenic bacteria in maize (*Zea mays* L.) seeds. *Interciencia* 26(3): 108-112.
Clavibacter michiganensis subsp. *nebraskensis**	Transmission at 1.6%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. Rocheford, T.R., A.K. Vidaver, C.O. Gardner, and D.L. Armbrust. 1985. Effect of wind generated sand abrasion on infection of corn (*Zea mays* L.) by *Corynebacterium michiganense* ssp. *nebraskense*. (Abstr.). *Phytopathology* 75: 1378.
*Pantoea stewartii**	Transmitted at between 0.05% and 30% depending on genotype. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.

Table 3. Maize pathogens..... cont'd.

FUNGI	
*Acremonium maydis**	Transmitted at up to 11%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp. Mohamed, H.A., W.E. Ashour, A.R. Sirry, and S.M. Fathi. 1967. Fungi carried by corn seed and their importance in causing corn diseases in the United Arab Republic. *Plant Disease Reporter* 51: 53-56. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Acremonium strictum	Transmitted at up to 40%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Cochliobolus heterostrophus (Bipolaris maydis, Helminthosporium maydis)	Transmitted at up to 99%. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Lasiodiplodia theobromae (Botryodiplodia theobromae)	Transmitted at up to 90%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Cochliobolus carbonum	Transmission not proven, but causes problems in seed germination and storage. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Fusarium culmorum	Transmitted at up to 38% McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Gibberella moniliformis	Transmitted at up to 100%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Gibberella zeae	Transmitted at up to 60%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. Foley, D.C. 1983. Effect of symptomless fungal infection of maize seed on germination in the presence and absence of *Pythium debaryanum. Proceedings of the Iowa Academy of Science* 90(4): 147-149.
Glomerella graminicola	Transmitted at from 9% up to 50%. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp. Warren, H.L. 1977. Survival of *Colletotrichum graminicola* in corn kernels. *Phytopathology* 67(2): 160-162.
Khuskia oryzae (Nigrospora oryzae)	Transmitted at up to 13%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
*Peronosclerospora maydis**	Transmitted only by fresh seed. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.

Table 3. Maize pathogens..... cont'd.

*Peronosclerospora philippinensis**	Transmitted at up to 11% by fresh seed. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
*Peronosclerospora sacchari**	Transmitted at up to 100%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Peronosclerospora sorghi	Transmitted only by fresh seed. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Phaeocytosporella zeae	Transmission proven under laboratory conditions only. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Sclerophthora macrospora	Transmitted at from 0.6% up to 60%. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
*Sclerophthora rayssiae var. zeae**	Transmission lower than 1%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. Putnam, M.L. 2007. Browne stripe downy mildew (*Sclerophthora rayssiae var. zeae*) of maize. Online. Plant Heath Progress doi:10.1094/PHP-2007-1108-01-DG. Singh, R.S., M.M. Joshi, and H.S. Chaube. 1968. Further evidence of the seed borne nature of corn downy mildews and their possible control with chemicals. *Plant Disease Reporter* 52: 446-449.
Sclerospora graminicola	Transmitted only by fresh seed. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International.
Setospaheria turcica	Transmission suspected but only confirmed by one study. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Setosphaeria rostrata	Transmissible, but no data on rate of transmission. Anahosur, K.H., and A. Sivanesan. 1978. *Setosphaeria rostrata. IMI Descriptions of Fungi and Bacteria* 59, Sheet 587.
Sphacelotheca reiliana	Seed-borne only, but infection can originate from spores present on the seed surface. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Stenocarpella maydis	Transmitted at up to 66.7%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
Ustilago maydis	Seed-borne only, but infection can originate from spores present on the seed surface. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.
NEMATODES	
*Heterodera zeae**	Cysts can be mixed and transported with seed. CAB International. 2007. *Crop Protection Compendium, 2007 Edition.* Wallingford, UK: CAB International.

* Quarantined under Norma Oficial Mexicana NOM-018-FITO-1995.

Table 4. Wheat and triticale pathogens transmitted by seed, with transmission levels and sources.

VIRUSES	
Barley stripe mosaic virus	Transmitted at up to 70%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition*. Wallingford, UK: CAB International. Maramorosch, K., and K.F. Harris (eds.). 1981. *Plant Diseases and Vectors: Ecology and Epidemiology*. New York: Academic Press. pp. 293-317.
Wheat streak mosaic virus	Transmission ranges from 0.2 to 1.5%. Roger, A.C., B.A. Coutts, A.E. Mackie, and G.I. Dwyer. 2005. Seed transmission of wheat streak mosaic virus shown unequivocally in wheat. *Plant Disease* 89: 1048-1050.
BACTERIA	
Pseudomonas fuscovaginae	Transmission proven but level not established. Duveiller, E., and C. Martinez. 1990. Seed detection of *Pseudomonas fuscovaginae* in wheat. *Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent* 55(3a): 1047-1053.
Pseudomonas syringae pv. *atrofaciens*	Transmission proven but level not established. Duveiller, E., L. Fucikovsky, and K. Rudolph (eds.). 1997. *The Bacterial Diseases of Wheat: Concepts and Methods of Disease Management.* Mexico, D.F.: CIMMYT. p. 56.
Rathayibacter tritici	The bacterium is carried with the seed in infected galls of *Anguina tritici.* CAB International. 2007. Crop Protection Compendium, 2007 Edition. Wallingford, UK: CAB International.
Xanthomonas translucens pv. *undulosa*	Transmitted at up to 25%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition*. Wallingford, UK: CAB International.
FUNGI	
*Alternaria triticina**	Seed-borne inoculum plays a major role in disease perpetuation, but level of transmission not established. CAB International. 2007. *Crop Protection Compendium, 2007 Edition*. Wallingford, UK: CAB International.
*Cephalosporium gramineum**	Transmission between 0.18 and 0.55%. Murray, T.D. 2006. Seed transmission of *Cephalosporium gramineum* in winter wheat. *Plant Disease* 90: 803-806.
Claviceps purpurea	Up to 70% of the seed can be replaced by sclerotia. CAB International. 2007. *Crop Protection Compendium, 2007 Edition*. Wallingford, UK: CAB International.
Cochliobolus sativus	Transmitted at up to 90%. Goulart, A.C.P. 1996. Transmission of *Bipolaris sorokniana* from seeds to wheat coleoptiles. *Summa Phytopathologica* 22(1): 5-9.
Gibberella avenacea	Transmisson from seed to seedlings proven under laboratory conditions. Porta-Puglia, A., and S. Santorelli. 1994. Diseases of wheat transmissible by seed. *Sementi Elette* 40(5): 35-38.
Gibberella zeae	Transmission from 55 to 94%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition*. Wallingford, UK: CAB International.
Monographella nivalis	Rate of transmission unclear, although seed-borne inoculum is the main cause of seedling blight and reduced germination. CAB International. 2007. *Crop Protection Compendium, 2007 Edition*. Wallingford, UK: CAB International.
Phaeosphaeria nodorum (Stagonospora nodorum)	The relationship between seed infection and disease incidence on upper leaves is not linear, but in some cases transmission can reach 40%. CAB International. 2007. *Crop Protection Compendium, 2007 Edition*. Wallingford, UK: CAB International.
Pyrenophora tritici-repentis (Helminthosporium tritici-repentis)	Seed transmission at up to 92% in vitro and 60% in potting soil outdoors. Fernandez, M.R., R.M. DePauw, J.M. Clarke, and L.P. Lefkovitch. 1996. Red smudge in durum wheat reduces seedling vigour. *Canadian Journal of Plant Science* 76(2): 321-324.

Table 4. Wheat and triticale.... cont'd.

Magnaporthe grisea	Different levels of transmission depending on environmental conditions at sowing. CAB International. 2007. *Crop Protection Compendium, 2007 Edition*. Wallingford, UK: CAB International. Urashima, A.S., C.R.F. Grosso, A. Stabili, E.G. Freitas, C.P. Silva, D.C.S. Netto, I. Franco, J.H. Merola Bottan. 2009. Effect of *Magnaporthe grisea* on seed germination, yield,and quality of wheat. In G.L. Wang and B. Valent (eds.), *Advances in Genetics, Genomics, and Control of Rice Blast Disease*. Dordrecht: Springer Netherlands. pp. 267-277.
Sclerophthora macrospora	Evidence of transmission under laboratory conditions. Bains, S.S., and J.S. Jhooty. 1985. Seed transmission of *Sclerophthora macrospora* in wheat. *Seed Research* 13(2): 154-156.
*Tilletia controversa**	Bunted grains release spores into soil, where they can germinate and infect new seedlings. Grey, W.E., D.E. Mathre, J.A. Hoffmann, R.L. Powelson, and J.A. Fernández. 1986. Importance of seedborne *Tilletia controversa* for infection of winter wheat and its relationship to international commerce. *Plant Disease* 70(2): 122-125. Wilcoxson, R.D., and E.E. Saari (eds.). 1996. *Bunt and Smut Diseases of Wheat: Concepts and Methods of Disease Management*. Mexico, D.F.: CIMMYT.
*Tilletia indica**	Bunted grains release spores into soil, where they can germinate and infect plants at the flowering stage. Bains, S.S., and H.S. Dhaliwal. 1989. Release of secondary sporidia of *Neovossia indica* from inoculated wheat spikes. *Plant and Soil* 115(1): 83-87. Wilcoxson, R.D., and E.E. Saari (eds.). 1996. *Bunt and Smut Diseases of Wheat: Concepts and Methods of Disease Management*. Mexico, D.F.: CIMMYT.
Tilletia laevis	Bunted grains release spores into soil, where they can germinate and infect new seedlings. Wilcoxson, R.D., and E.E. Saari (eds.). 1996. *Bunt and Smut Diseases of Wheat: Concepts and Methods of Disease Management*. Mexico, D.F.: CIMMYT.
Tilletia tritici	Bunted grains release spores into soil, where they can germinate and infect new seedlings. Wilcoxson, R.D., and E.E. Saari (eds.). 1996. *Bunt and Smut Diseases of Wheat: Concepts and Methods of Disease Management*. Mexico, D.F.: CIMMYT.
Urocystis agropyri	Seed infestation with dry teliospores was shown to create an artificial epidemic of flag smut. Rewal, H.S., Goel R.K., and J.S. Jhooty. 1986. Seed and soil inoculation in relation to the incidence of flag smut in wheat. *Indian Phytopathology* 39(4): 599-600.
Ustilago tritici	A 100% correlation has been found between infected seed and incidence of loose smut in the field. CAB International. 2007. *Crop Protection Compendium, 2007 Edition*. Wallingford, UK: CAB International.
NEMATODES	
*Anguina tritici**	The principal means of dispersion is by wheat seed containing infected galls. Luc, M., R.A. Sikora, and J. Bridge. 1990. Nematode parasites of cereals. In: *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. Wallingford, UK: CAB International. pp. 109-136.
Heterodera avenae	Cysts borne externally among grains. CAB International. 2007. *Crop Protection Compendium, 2007 Edition*. Wallingford, UK: CAB International.
Heterodera zeae	Cysts borne externally among grains. CAB International. 2007. *Crop Protection Compendium, 2007 Edition*. Wallingford, UK: CAB International.

* Quarantined under Norma Oficial Mexicana NOM-017-FITO-1995.

Appendix 3: Letter of commercial value template

[Letterhead of Issuing Institution/Company]
[En papel membretado de la institución o empresa]

Date (Fecha): ____________

Commercial Letter
Carta Comercial

To whom it may concern:
A quien corresponda:

Box(es) containing ________ kg of (indicate which species) seed samples donated for research purposes, with no commercial value and an estimated value "for customs purposes only" of: _______*

Caja(s) que contienen _________ kg de muestras de semilla de (indicar cual especie) donadas para uso experimental sin valor comercial, con un valor aproximado "solo para propositos aduanales" de: _______*

Shipper's Signature
Firma del Consignatario

____

* **Amount suggested is US $0.50 per kg of seed, but do not declare a total amount lower than US $1.00 or higher than US $100.00.**

* Se sugiere la cantidad de US $0.50 por cada kilo de semilla; no declarar una cantidad total inferior a US $1.00 o que exceda US $100.00.

Appendix 4: Certificate of origin template

[On letterhead of shipping institution/company]
(En papel membretado de la institución o empresa)

CERTIFICATE OF ORIGIN
CERTIFICADO DE ORIGEN____

To whom it may concern:
A quien corresponda:

Date (Fecha):____________________

DESCRIPTION: Seed of : indicate the species
DESCRIPCION: Semilla de indicar la especie

____

AMOUNT: (in g or kg):
CANTIDAD: (en g o kg):

____

ORIGIN: (Location of production field(s): site, state or province, country)
ORIGEN: (Localización del campo de producción: lugar, estado o provincia, país)

____

PURPOSE OR USE: Experimental use only
PROPOSITO O USO: Exclusivamente para uso experimental

____

VALUE: No commercial value
VALOR: Sin ningún valor comercial

____

REMARKS: Fumigated, treated, etc. (be specific, e.g., Vitavax)
NOTAS: Fumigada, tratada, etc. (especificar, por ejemplo, Vitavax)

____

AUTHORIZED SIGNATURE:
FIRMA AUTORIZADA: ______________________________

(Type name and designation of person signing,
and of the shipping institution/company)
(Poner a máquina el nombre y designación del
signatario, y de la institución o empresa)____

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