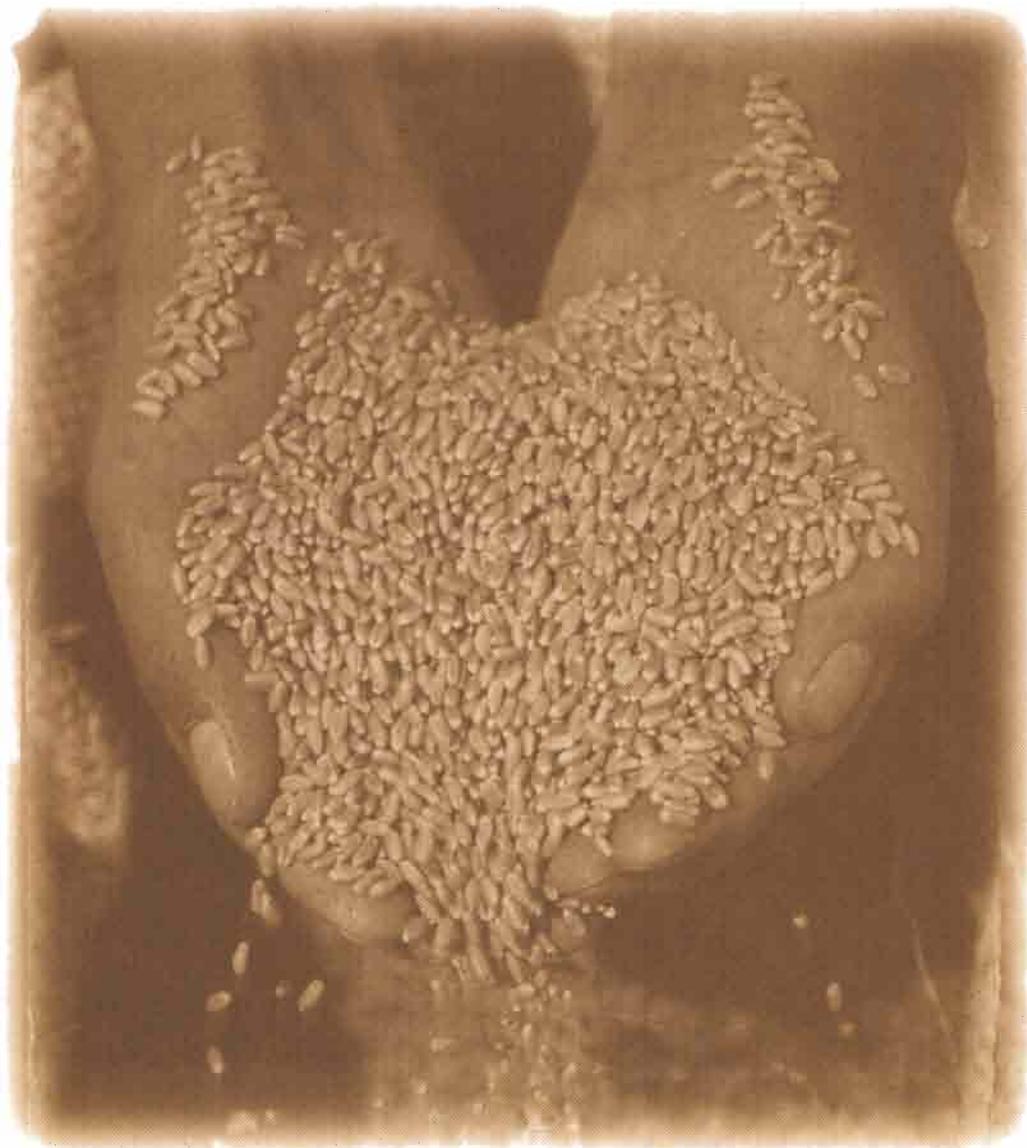


SEED HEALTH:

RULES AND REGULATIONS FOR THE SAFE MOVEMENT OF GERMPLASM

M. Mezzalama, L. Gilchrist, and A. McNab

Seed Health Unit



 **CIMMYT**[®]

INTERNATIONAL MAIZE AND WHEAT IMPROVEMENT CENTER

**SEED HEALTH:
RULES AND REGULATIONS FOR THE
SAFE MOVEMENT OF GERMPLASM**

**M. Mezzalama, L. Gilchrist,
and A. McNab**

Seed Health Unit



CIMMYT®

CIMMYT® (www.cimmyt.cgiar.org) is an internationally funded, nonprofit scientific research and training organization. Headquartered in Mexico, CIMMYT works with agricultural research institutions worldwide to improve the productivity, profitability, and sustainability of maize and wheat systems for poor farmers in developing countries. It is one of 16 food and environmental organizations known as the Future Harvest Centers. Located around the world, the Future Harvest Centers conduct research in partnership with protecting natural resources. The centers are supported by the Consultative Group on International Agricultural Research (CGIAR) (www.cgiar.org), whose members include nearly 60 countries, private foundations, and regional and international organizations. Financial support for CIMMYT's research agenda also comes from many other sources, including foundations, development banks, and public and private agencies.

F U T U R E Future Harvest,® builds awareness and support for food and environmental research for a **HARVEST** world with less poverty, a healthier human family, well-nourished children, and a better environment. It supports research, promotes partnership, and sponsors projects that bring the results of research to rural communities, farmers, and families in Africa, Asia, and Latin America (www.futureharvest.org).

© International Maize and Wheat Improvement Center (CIMMYT) 2001. All rights reserved. The opinion expressed in this publication are the sole responsibility of the authors. The designations employed in the presentation of materials in this publication do not imply the expression of any opinion whatsoever on the part of CIMMYT or its contributory organizations concerning the legal status of any country, territory, city or area, or of its authorities, or concerning the delimitation of its frontiers or boundaries. CIMMYT encourages fair use of this material. Proper citation is requested.

Correct citation: Mezzalama, M., L. Gilchrist, and A. McNab. 2001. *Seed Health: Rules and Regulations for the Safe Movement of Germplasm*. Mexico, D.F.: CIMMYT.

ISBN: 970-648-067-6

AGROVOC descriptors: Wheat; Maize; Barley; Triticale; Seed certification; Seed testing; Seed treatment; Health policies; Mexico

AGRIS category codes: F03 Seed Production
F01 Crop Husbandry

Dewey decimal classification: 631.521

Printed in Mexico.

Seed Health Procedures for Incoming Seed

Required Documents

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.

Ordinary permit for crop seed entering Mexico

On 18 July 1989 the Mexican Government recognized CIMMYT as an international center with diplomatic rights. Despite this recognition, CIMMYT was not exempted from abiding by Mexican phytosanitary laws regarding the importation of germplasm into the country.

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 wheat, triticale, barley, or maize seed. The import permit application must be accompanied by bibliographic references supporting the presence of all seed-borne and seed-transmitted pathogens in the seed's country of origin.

An ordinary seed import permit normally takes 2-3 weeks to be granted, and remains valid for six months after issuance. Once the import permit is granted, CIMMYT will send a copy to the exporting cooperator. Instructions outlining proper importation 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 documents that must accompany seed destined for importation to Mexico are (cf. Appendix):

- ◆ A copy of the seed importation permit granted by the Mexican Government specifically for the country of origin. The original is kept at CIMMYT headquarters;
- ◆ The original certificate of origin;
- ◆ The original commercial letter of value;
- ◆ The original 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 seeds. The originals must be sent separately but concurrently with the seed shipment. It is very important that the originals be sent separately, because seed boxes are frequently opened before arriving in Mexico and the documents taken off the boxes. If the original importation documents are lost or misplaced before arriving in Mexico, the Mexican authorities will not release the shipment.

All seed shipments and the envelope containing the original documents must be addressed to:

CIMMYT Int.
Care of: Mr. Humberto Chavez Maldonado/COSACE
Oriente 180 no. 407
CP 15500 Mexico, D.F.
Tel/Fax (01) 55718787
MEXICO

Put the name of the consignee **inside** the box. Senders must put their complete address, including the ZIP CODE, on all documents. The ZIP CODE has become crucial for custom procedures due to a new regulation.

Special permission for importing crop seed into Mexico

A special import permit is required in the following cases:

- ◆ Untreated seed destined for laboratory or greenhouse analysis
- ◆ Vegetative tissue
- ◆ Fungal cultures or isolates

Permit applications for importing the above types of materials must be fully and carefully justified, reporting the material's precise place and country of origin, description (variety, weight, and any other useful information), 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. Otherwise, CIMMYT will have to provide additional information, and the application process will restart all over again. This type of permit is valid **ONLY** for the specified material. If a shipment is different in content or quantity (even by only 1 g of seed or 1 leaf more) from that specified on the permit, it will be confiscated.

Seed Health Testing

Role of CIMMYT's Seed Health Unit (SHU) laboratory

The DGSV authorities officially recognize several private and public laboratories in Mexico for seed health testing. All imported seed must be checked by one of these laboratories before release, at considerable expense (about US\$50 per test).

The CIMMYT SHU has been officially authorized by DGSV to conduct these importation inspections and scrutinize all phytosanitary tests on seed introductions coming into Mexico to CIMMYT. A DGSV official inspector is assigned exclusively to CIMMYT to assist with thorough and timely seed inspection and importation.

Given this authorized regulatory function of the SHU, seed importation into Mexico for experimental purposes is facilitated as follows:

- ◆ Seed sampling and inspection at the point of entry (e.g., Mexico City airport) is avoided, since it will be done at CIMMYT by the internal inspector. This ensures that the box will come into CIMMYT closed and as originally packed.
- ◆ Inspection and clearance time are expedited.
- ◆ Thorough phytosanitary procedures are followed.

Laboratory testing procedures

All seed brought into CIMMYT, without exception, must pass through the Seed Health Unit. If seed is delivered directly to a program, packages must NOT be opened and should be taken to the SHU laboratory immediately.

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, the inspector will decide on sample size and the laboratory tests to be conducted.

Consignees are informed that the SHU has received the introduction by 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, SHU registration number). Data and results of seed introductions are also entered in a public folder on CIMMYT's Web page. www.cimmyt.org

Wheat pathogens considered of quarantine interest in Mexico are included in the federal law Norma Oficial Mexicana NOM-017-1995, and maize pathogens in the Norma Oficial Mexicana NOM-018-1996, established by Mexico's Ministry of Agriculture, Livestock, and Rural Development (Secretaría de Agricultura, Ganadería y Desarrollo Rural).

However, CIMMYT also feels a strong responsibility to prevent the importation of any organism considered potentially harmful to these crops by the SHU (Tables 3 and 4 in the Appendix). Furthermore, since weed species have recently been quarantined by the federal law Norma Oficial Mexicana NOM-043-FITO1999, the SHU also ensures that shipments are cleaned of weed seeds before planting.

CIMMYT SHU personnel will conduct the following tests on wheat seeds, as listed in Table 1, and on maize seeds, as listed in Table 2.

Seed wash filter test. This test reveals the presence of spores of bunts (*Tilletia indica*, *Tilletia controversa*, quarantined by NOM-017-FITO-1996), smuts (*Urocystis agropyri*, *U. occulta* var. *tritici*, *Ustilago avenae*, *U. hordei*, *U. nuda*, *U. tritici*, *U. maydis*), sclerotia (*Claviceps purpurea*), oospores (*Peronosclerospora* and *Sclerophthora* spp.), nematodes (*Anguina tritici* quarantined by NOM-017-FITO-1996, and *Heterodera zea*, quarantined by NOM-018-FITO-1995). This test takes anywhere from a few hours to a few days, depending on the size of the sample.

Freeze blotter test. This test reveals the presence of imperfect fungi carried by seed and takes two weeks. *Alternaria triticina* on wheat is quarantined by NOM-017-FITO-1996; *Acremonium* (*Cephalosporium*) *maydis* on maize by NOM-018-FITO-1995.

Greenhouse germination test. The aim of this test is to obtain the expression of seed-borne bacterial or viral pathogens and to check seed viability. It takes three weeks. *Pseudomonas syringae* pv. *atrofaciens* is quarantined by NOM-017-FITO-1996. *Burkholderia* (*Pseudomonas*) *andropogonis*, *Clavibacter michiganensis* pv. *nebraskensis*, and *Pantoea stewartii* are quarantined by NOM-018-FITO-1995. The

latter two pathogens have been reported in Mexico (Allende-Molar, 1996; Avila et al., 1999) and are considered A2 quarantined pests, though NOM-018 has not been updated. Wheat streak mosaic virus is quarantined by NOM-018-FITO-1995.

If symptoms appear on seedlings, ELISA test for *P. stewartii* and/or a pathogenicity test for the identification of the causal pathogen will be carried out.

Downy mildew detection on maize seed coming from East and Southeast Asia. This test is conducted to detect the presence of quarantined downy mildew pathogens *Sclerospora maydis*, *Sclerospora* (*Peronosclerospora*) *philippinensis*, *Peronosclerospora sacchari*, and *Sclerophthora rayssiae* var. *zeae* (NOM-018-FITO-1995).

Results

The official inspector uses data from the SHU to judge whether a shipment should be:

Approved for release, given that

- ◆ The seed came in properly disinfected and is pathogen-free.
- ◆ The seed was not properly disinfected at origin but it is free of pathogens of quarantine concern in Mexico. In this case, the SHU will recommend the appropriate treatment to the program.

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

Rejected, given that

- ◆ The seed carries a pathogen(s) quarantined by Mexico's plant health authorities. If the material is very valuable or rare, it will remain under strict SHU control. It will be sown under restricted conditions and continually monitored to eliminate the pathogen and prevent dispersal. **After this process, the seed can be released.** However, if the risk cannot be eliminated, the seed will be incinerated or returned to the sender.

Every month the official inspector 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 for release described above may be taken away, after being released, by a program-designated assistant only and planted exclusively and without exception in the introduction blocks. The purpose of this procedure is to monitor, detect, and destroy any pathogen that may have not been detected by laboratory tests.

Wheat introductions must be sown in Ciudad Obregon in the quarantine plots of Block 710, in Toluca in Blocks A₂ and A₃, and in El Batan, in Block E₉ and F₉. Maize introductions must be sown in Tlatizapan in an introduction block that may change from year to year, and in El Batan in Block E10.

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 SHU 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. The programs should also indicate whether they intend to do disease evaluation.

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

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

Seed Health Procedures for Outgoing Seed

Required Documents

All wheat, triticale, barley, and maize germplasm exported by CIMMYT in an international nursery or miscellaneous shipment must be accompanied by three documents:

- ◆ An international phytosanitary certificate issued by the Government of Mexico
- ◆ A CIMMYT health certificate (in either English or Spanish) issued by the SHU
- ◆ Declaration of value

Sanidad Vegetal issues international phytosanitary certification by randomly checking seed packets. Certification by CIMMYT is based on examination of whole seed and the use of seed washing, greenhouse tests, incubation, and serological tests conducted by the SHU.

CIMMYT maize, barley, triticale, and wheat seed is dispatched as clean as possible, to avoid the spread of seed-borne plant diseases and any risk of 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 laws regulate pests all over the world as 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, barley, triticale, and wheat seed that is shipped must be free from A1 and A2 pests.

Laboratory tests conducted by the SHU to detect possible seed-borne and seed-transmitted pathogens are listed in Tables 1 and 2. Other tests are conducted upon request if the country of destination has special requirements.

Wheat

Seed multiplication in Mexicali, Baja California, Mexico

The Mexicali area, in northwestern Mexico, has officially been declared free from Karnal bunt (KB) according to the Diario Oficial de la Federación published on 15 December 1997, following the instructions given in federal Mexican law NOM-001-FITO-1995. 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 from Mexico.

Seed planting procedures in Mexicali

Wheat, barley, and triticale germplasm to be included in an international nursery should be planted in plots assigned for that purpose at El Batán Experiment Station, an area free of *Tilletia indica*, causal agent of Karnal bunt.

Table 1. Seed health testing on wheat, triticale, and barley.

NAME OF THE NURSERY	TEST APPLIED	PATHOGEN DETECTED	DURATION OF THE TEST	
Outgoing seed International Wheat Nursery International Triticale Nursery International Barley Nursery Miscellaneous nurseries w, tcl, b International Wheat Nursery	Filter ELISA Blotter Greenhouse germination Immunofluorescence	<i>Tilletia</i> spp. BSMV Fungi Bacteria-Virus Seed viability <i>Xanthomonas translucens</i> <i>pv. undulosa</i>	3 hrs 24 hrs 3 weeks 3 weeks few hours	
	More tests carried out according to requests by country of destination			
				QUARANTINED IN MEXICO (A1) *
Incoming seed	Blotter Greenhouse germination Filter	Fungi Bacteria Virus <i>Tilletia</i> spp. Nematodes <i>Ustilago</i> spp. <i>Urocystis</i> spp.	3 weeks 3 weeks few hrs	<i>Alternaria triticina</i> <i>Pseudomonas syringae</i> <i>pv. artrofaciens</i> <i>Tilletia indica</i> , <i>T. controversa</i> <i>Anguina tritici</i>

* According to Norma Oficial Mexicana NOM-017-FITO-1996. A1 pest = quarantine pest not present in that area. A2 pest = quarantine pest present in that area but not widely distributed and being officially controlled.

Table 2. Seed health testing on maize.

NAME OF THE NURSERY	TEST APPLIED	PATHOGEN DETECTED	DURATION OF THE TEST	
Outgoing seed International Maize Nurseries Certification Maize Miscellaneous Nurseries	ELISA Blotter Greenhouse germination More tests carried out according to requests by country of destination	<i>Pantoea stewartii</i> MDMV (depending on greenhouse results) Fungi Bacteria - Virus	24 hrs 24 hrs 2 weeks 3 weeks	
				QUARANTINED IN MEXICO (A1) *
Incoming seed	Blotter Greenhouse germination Filter Downy mildew detection for germplasm coming from South and Southeast Asia	Fungi Bacteria Virus Nematodes Oospores Smuts and bunts	2 weeks 3 weeks 3 hrs 24 hrs	<i>Cephalosporium maydis</i> <i>Pantoea stewartii</i> (A2**) <i>Pseudomonas andropogonis</i> <i>Clavibacter michiganensis</i> <i>pv. nebraskensis</i> Wheat streak mosaic virus <i>Heterodera zea</i> <i>Peronosclerospora</i> spp. <i>Sclerophthora</i> spp. <i>Sclerospora maydis</i> <i>Sclerospora (Peronosclerospora) philippinensis</i> <i>Peronosclerospora sacchari</i> <i>Sclerophthora rayssae</i> f.sp. <i>zea</i>

* According to Norma Oficial Mexicana NOM-018-FITO-1995. A1 pest = quarantine pest not present in that area. A2 pest = quarantine pest present in that area but not widely distributed and being officially controlled.

During the crop cycle, plots will be sprayed with the systemic fungicide propiconazole (TILT) every 10 days, from spike emergence to the end of grainfilling, to protect the plots from aerial infections of *T. indica*.

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

During the crop cycle in Mexicali, the germplasm is constantly monitored to produce the highest quality seed. It is sprayed 2-3 times with propiconazole following the same method as before. 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 fields in Mexicali are required to bring clean clothes and shoes to wear and to wash their vehicles before arrival.

The germplasm is inspected at the end of the cycle, close to harvest time. Randomly sampled spikes are collected, individually threshed, and visually examined to detect potential seed health problems. The seed is then tested following procedures described in the Appendix. If the filter test results are negative, each line is individually harvested.

The harvested seed is placed in new bags that have been appropriately tagged. The bags and tags used at harvest must be shipped by air from KB-free areas to Mexicali. Harvested seed is appropriately packed and transported to CIMMYT headquarters in El Batán, State of Mexico, 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 coming into 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 to confirm that the area is free of any contaminants. All areas and all procedures are under zero tolerance for KB.

Lines received in the International Nurseries area can now be selected by the program head for inclusion in an international trial. The selected lines are cleaned by sifting through screens to eliminate weed seed and large debris. Before international distribution, every line of every nursery is washed and sampled for the filter test using the following procedure:

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

1. placed under high water pressure + 10 ml of Tween 20 for 6 minutes at 50-60 pounds/inch²;
2. immersed for 3 minutes in 1.5% sodium hypochlorite solution; and
3. rinsed for 2 minutes under high water pressure.

After washing, a seed sample (about 50 g) is taken from each tray and sent to the SHU. Depending on the amount of seed from the line, the sample is sub-sampled and one or more filter tests for the presence of *T. indica* and *Tilletia* spp. teliospores are carried out as described in the Appendix.

While the tests are being conducted, the remaining seed, after washing, is treated with a slurry containing chlorothalonil (Daconil 2787, 2 g/kg) and carboxin+captan (Vitavax 300, 3 g/kg), plus an adhesive (Pellistac). The seed is oven dried at 30 °C. If the filter tests results are positive for the presence of *T. indica* teliospores, the line is discarded, even if it has already been treated.

Washed, treated, and dried seed is placed in envelopes, and the nursery is assembled for shipping to CIMMYT cooperators. Seed selected by visitors during the Obregon cycle (see “Miscellaneous shipments” below) must follow this same procedure.

Miscellaneous shipments

Occasional nurseries called “miscellaneous” or “special” nurseries that have not been multiplied in Mexicali are also part of International Nurseries. Any seed that was not multiplied in Mexicali merits special care for several reasons:

- ◆ Seed produced in El Batan or Toluca is of lower quality, mainly due to environmental conditions (high rainfall areas) and higher disease incidence.
- ◆ The crop is not always sprayed with fungicide during the cycle as it is in Mexicali.
- ◆ Though El Batan and Toluca are KB-free areas, they have not been officially recognized as such.

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

Accurate laboratory examination is required for Karnal bunt and other diseases of quarantine concern. All lines must be delivered to the lab for inspection and the filter wash test. It is up to SHU personnel to 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, as for International Nursery distribution.

Detection of other seed-borne pathogens of wheat

Tests carried out by the SHU on wheat, triticale, and barley are given in Table 1.

Maize

Most maize seed destined for international distribution is produced in CIMMYT’s maize experiment station in Tlaltizapan (State of Morelos), a site with very low disease incidence. During the crop cycle, nurseries are periodically inspected in the field; plants showing symptoms are discarded. After harvest, all seed is carefully checked for the presence of seed-transmitted pathogens of quarantine interest in countries of destination.

The Maize Program submits to the SHU three groups of germplasm for testing: International Nurseries, miscellaneous nurseries, and materials for certification. Tests carried out by the SHU on maize seed are given in Table 2. Maize seed is treated before shipment with a slurry containing acephate (organophosphoric insecticide), captan, and an adherent.

Laboratory Testing on Maize, Wheat, Triticale, and Barley

Greenhouse germination test

Greenhouse germination is an important test for evaluating seed viability and detecting the presence or absence of seed-transmitted pathogens. This test is conducted on a 50-seed sample of each line in a nursery. Further investigation is done if symptoms appear on seedlings.

Seed wash filter test

This test is applied mainly for the detection of *Tilletia indica* and *Tilletia* spp. teliospores, mildew oospores, and smut spores following the procedure described in the Appendix. This test can be carried out either on composite seed samples or line by line.

Freeze blotter test

This test is applied for the detection of imperfect fungi on 40-50 seeds from every entry in each nursery.

ELISA

This test is applied for the detection of 1) barley stripe mosaic virus, which is on EPPO's A2 quarantine list (2001); 2) *Pantoea stewartii*, which is on EPPO's A2 quarantine list (2001), and is an A1 pest in more than 100 countries (Block et al., 1998), and an A2 pest in Argentina, Brazil, Peru, Kenya, Nigeria, Zimbabwe, Bangladesh, and the Philippines, and 3) maize dwarf mosaic virus, which is an A2 pest in Zimbabwe. Composite samples of every 10 lines in a nursery are tested. In case of a positive response, the 10 lines forming the sample are individually re-tested. Lines that test positive are discarded.

Immunofluorescence

This is a sero-diagnostic assay for the detection on wheat seed of *Xanthomonas translucens* pv. *undulosa*, which is an A1 pest in Egypt, Saudi Arabia, and Turkey (on any grass seed) (CABI 2000) and is on EPPO's A2 quarantine list (2001). Composite samples of every 10 lines in a nursery are tested. In case of a positive result, the 10 lines are individually checked. Lines that test positive are discarded.

References

- Allende-Molar, R. 1996. *Clavibacter michiganense* subsp. *nebraskense* agente causal de la marchitez y tizón bacteriano del maíz en el estado de Sinaloa. Master of Science Thesis, Universidad Autónoma de Sinaloa, Facultad de Agronomía, Culiacán, Sinaloa, México.
- Avila, G., L. Fucikovsky, D. Jeffers, D. De La I de Bauer, M. Lopez, and J. Molina. 1999. Distribución de *Pantoea (Erwinia) stewartii* en maíz en Valles Altos de México. XLV Reunión Anual del PCCMCA: Memorias de Resúmenes 58.
- Block, C.C., J.H. Hill, and D.C. McGee. 1998. Seed transmission of *Pantoea stewartii* in field and sweet corn. *Plant Disease* 82:775-780.
- CAB International. 2000. Crop Protection Compendium, Global Module. 2nd edition. Wallingford, UK: CAB International.
- EPPO, 2001. <http://www.eppo.org/QUARANTINE/lists.html#a1>

Acknowledgments

The authors wish to thank Tom Payne and Dan Jeffers for their collaboration in producing this section of the document.

Rules for Storing Seed under Conditions of Zero Tolerance for Karnal Bunt Teliospores

Preventive Seed Health Procedures to be Applied before Placing Seed in Storage

Warehouse hygiene

Warehouses should be washed and cleaned regularly. Floors and surfaces must be disinfected with a 1% sodium hypochlorite solution. Slide traps must be positioned in every chamber, room, or common environment to check contamination.

Seed testing before storage

Seed to be placed in storage should have been multiplied in areas free of Karnal bunt (KB) and subjected to the following SHU procedures:

- ◆ Seed is washed in a 1.2% sodium hypochlorite solution for 3 min. A sample is then taken and tested using the filter procedure in the SHU.
- ◆ To avoid reducing seed viability, seed should NOT be treated with fungicide after washing. Furthermore, for health reasons, CIMMYT staff should NOT work with fungicide-treated seed unless absolutely necessary.
- ◆ Seed must be checked for KB before shipping to cooperators.

Seed is stored in metal containers to avoid contamination. Upon storage, the SHU 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 whenever they go inside the storeroom, staff must wear sneakers and clothing (aprons or overalls) reserved specifically for this purpose. Lab 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% 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 (the kind used in swimming pools).

Rules for Moving 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 federal law NOM-001-FITO-1995. Therefore:

- ◆ All seed produced in the CIANO-CIMMYT experiment station in Ciudad Obregon, Sonora, and coming into El Batan and Toluca experiment stations must be inspected on arrival by a technician accredited by DSGV;
- ◆ All seed shipments must be accompanied by a seed health certificate authorizing seed movement within Mexico (*Certificado fitosanitario para la movilización nacional*), and
- ◆ All seed must be treated.

Three technicians at CIMMYT headquarters are accredited every year by DSGV to release seed coming from Ciudad Obregon. However, it should be stressed that seed from Ciudad Obregon should be sown only in the El Batan and Toluca experiment stations.

Appendix

Filter wash 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, liquid laundry detergent can be used). Filter the wash water through 50- and 20-mm sieves. Large spores and debris will be caught on the 50-mm sieve, and *Tilletia indica* teliospores, with an average diameter of 25-40 μ m, will remain on the 20 mm sieve.

Rinse sieve with a water gun, catching the overflow in a beaker. Then pass the water through Whatman #1 filter paper placed in a Buchner funnel that is inserted into a flask attached to a vacuum pump. Whatman #1 filter paper will trap all structures smaller than 20 μ m.

After filtering the water, moisten the filter with a 3% KOH solution, and examine under a stereo microscope for the presence of spores. Mark any suspicious structures and confirm under a compound microscope.

List of pathogens that must accompany the application for an ordinary permit to import maize seed.

CUADRO 3. ENFERMEDADES DE MAIZ TRANSMITIDAS POR SEMILLA.

Table 3. Seed-transmitted maize pathogens.

PATOGENOS (Pathogens)	Presencia o ausencia en el país de origen (Presence/absence in country of origin)
VIRUS (Viruses)	
Maize chlorotic mottle virus**	
Maize dwarf mosaic virus**	
Maize leaf fleck virus (barley yellow dwarf virus)**	
Wheat streak mosaic virus*	
BACTERIAS (Bacteria)	
<i>Acidovorax avenae</i> subsp. <i>avenae</i> **	
<i>Burkholderia andropogonis</i> *	
<i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> *	
<i>Erwinia chrysanthemi</i> pv. <i>zeae</i> **	
<i>Pantoea agglomerans</i> **	
<i>Pantoea stewartii</i> *	
<i>Pseudomonas atrofaciens</i> pv. <i>zeae</i> **	
<i>Pseudomonas avenae</i> **	
<i>Pseudomonas marginalis</i> pv. <i>marginalis</i> **	
<i>Pseudomonas syringae</i> pv. <i>lapsa</i> **	
HONGOS (Fungi)	
<i>Acremonium maydis</i> *	
<i>Acremonium strictum</i> **	
<i>Bipolaris maydis</i> (<i>Helminthosporium maydis</i>)**	
<i>Botryodiplodia theobromae</i> **	
<i>Claviceps gigantea</i> **	
<i>Cochliobolus carbonum</i> **	
<i>Fusarium culmorum</i> **	
<i>Fusarium graminearum</i> **	
<i>Fusarium semitectum</i> **	
<i>Gibberella fujikuroi</i> **	
<i>Glomerella graminicola</i> **	
<i>Nigrospora oryzae</i> **	
<i>Peronosclerospora maydis</i> (<i>Sclerospora maydis</i>)*	
<i>Peronosclerospora philippinensis</i> *	
<i>Peronosclerospora sacchari</i> *	
<i>Peronosclerospora sorghi</i> **	
<i>Phaeocytoporella zeae</i> **	
<i>Pyricularia grisea</i> **	
<i>Sclerophthora macrospora</i> **	
<i>Sclerophthora rayssiae</i> var. <i>zeae</i> *	
<i>Setosphaeria turcica</i> **	
<i>Setosphaeria rostrata</i> **	
<i>Sphacelotheca reiliana</i> **	
<i>Stenocarpella maydis</i> **	
NEMATODOS (Nematodes)	
<i>Heterodera zeae</i> *	

* Señalados en la Norma de Cuarentena Mexicana (A1 quarantine pathogens).

** Agregados por el Laboratorio del CIMMYT (precautionary pathogens added by CIMMYT's SHU).

CUADRO 4. ENFERMEDADES DE CEREALES DE GRANO PEQUEÑO TRANSMITIDAS POR SEMILLA.
Table 4. Seed-transmitted pathogens of small-grain cereals.

PATOGENOS (Pathogens)	Presencia o ausencia en el país de origen (Presence/absence in country of origin)
VIRUS (Viruses)	
Barley stripe mosaic virus (Virus estriado de la cebada)**	
Brome mosaic virus (Mosaico estriado del bromo)**	
Wheat streak mosaic virus**	
BACTERIAS (Bacteria)	
<i>Bacillus megaterium</i> pv. <i>cerealis</i> **	
<i>Clavibacter michiganensis</i> subsp. <i>tessellarius</i> **	
<i>Erwinia rhapontici</i> **	
<i>Pseudomonas cichorii</i> **	
<i>Pseudomonas fuscovaginae</i> **	
<i>Pseudomonas syringae</i> pv. <i>atrofaciens</i> *	
<i>Pseudomonas syringae</i> pv. <i>coronofaciens</i> **	
<i>Pseudomonas syringae</i> pv. <i>syringae</i> **	
<i>Rathayibacter tritici</i> **	
<i>Xanthomonas translucens</i> pv. <i>translucens</i> **	
HONGOS (Fungi)	
<i>Alternaria triticina</i> *	
<i>Cephalosporium gramineum</i> **	
<i>Claviceps purpurea</i> **	
<i>Cochliobolus sativus</i> **	
<i>Cochliobolus spicifer</i> **	
<i>Cochliobolus victoriae</i> **	
<i>Gibberella avenacea</i> **	
<i>Gibberella zeae</i> **	
<i>Monographella nivalis</i> **	
<i>Mycosphaerella graminicola</i> (<i>Septoria tritici</i>)**	
<i>Phaeosphaeria avenaria</i> (<i>Stagonospora avenae</i> f. sp. <i>avenae</i>)**	
<i>Phaeosphaeria nodorum</i> (<i>Stagonospora nodorum</i>)**	
<i>Pyrenophora graminea</i> (<i>Helminthosporium gramineum</i>)**	
<i>Pyrenophora teres</i> (<i>Helminthosporium teres</i>)**	
<i>Pyrenophora tritici-repentis</i> (<i>Helminthosporium tritici-repentis</i>)**	
<i>Pyricularia grisea</i> **	
<i>Pyricularia oryzae</i> **	
<i>Rhynchosporium secalis</i> **	
<i>Sclerophthora macrospora</i> **	
<i>Tilletia caries</i> y <i>T. foetida</i> (<i>T. secalis</i>)**	
<i>Tilletia controversa</i> *	
<i>Tilletia indica</i> *	
<i>Urocystis agropyri</i> **	
<i>Urocystis occulta</i> var. <i>tritici</i> **	
<i>Ustilago avenae</i> **	
<i>Ustilago hordei</i> **	
<i>Ustilago nuda</i> **	
<i>Ustilago tritici</i> **	
NEMATODOS (Nematodes)	
<i>Anguina agrostis</i> **	
<i>Anguina tritici</i> *	
<i>Heterodera avenae</i> **	
<i>Heterodera latipons</i> **	
<i>Heterodera zeae</i> **	

* Señalados en la Norma de Cuarentena Mexicana (A1 quarantine pathogens).

** Agregados por el Laboratorio del CIMMYT (precautionary pathogens added by CIMMYT's SHU).

[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 _____ (maize,
wheat, barley, or whatever) 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 (maíz, trigo, cebada o lo que sea) donadas para uso experimental sin valor
comercial, con un valor aproximado "solo para propósitos 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.

[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: Wheat, triticale, barley, maize seed
DESCRIPCION: Semilla de trigo, triticale, cebada, maíz

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)** _____

ISBN: 970-648-067-6



INTERNATIONAL MAIZE AND WHEAT IMPROVEMENT CENTER

Apartado Postal 6-641, 06600 Mexico, D.F., MEXICO

www.cimmyt.org