

Seedborne Pathogens

S. F. Nome¹, Dora Barreto, Delia M. Docampo

Pathogens associated to the seed.

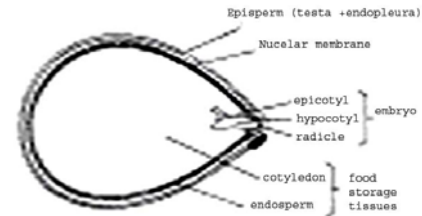
As an introduction we will remind some aspects of the morphology and anatomy of the seed, in order to understand in a better way the effect pathogens have in the seed and later, the plant originated from it.

The Seed

The seed consists of three basic parts: a) embryo, b) storage tissues and c) seed coat.

a) The embryo has a reproductive function, being capable of initiating cellular divisions and growth. It is the most important part of the seed. It is an axis that originates growth in two directions, with the objective of originating a root and a shoot. Usually, the embryo is very small, compared to other parts of the seed. It is like a miniature plant. It consists of a radicle, plumule, one or two cotyledons, and hypocotyl or epicotyl, depending on the type of plant.

b) Energy storage is localized in the cotyledons, in the endosperm or the perisperm. Cotyledons originate from the zygote and are part of the embryo. In many species, storage substances are localized in the cotyledons, and the embryo develops absorbing all the endosperm.



c) Episperm is the seed cover, consists of two layers, the testa and the endopleura. The outer layer is the testa; it can be stony, leathery, membranous or fleshy. Over the testa we can recognize: the hilum, scar or point of attachment of the seed to the funiculus, water penetrates easily through it; Micropyle, the point upon the seed at which was the orifice of the ovule through which the pollen tube enters; raphe, suture originated by the part of the funiculus that is fused along the side of the ovule. The endopleura is the inner layer, it is thin and generally whitish. Teguments, testa or protective covers delimit the seed. They are formed by one or more layers of cells originated from the ovule integuments and sometimes from the pericarp made from the walls of the ovary [1, 2].

¹ Instituto de Fitopatología y Fisiología Vegetal, INTA, Camino 60 Cuadras km %1/2 (5119), Córdoba, Argentina. sfnome@reidel.com.ar

Regarding the healthiness or pathology of the seed we will consider three aspects that allow us to understand processes associated to seed-borne diseases: infection of the seed, infection of the plant and steps that can be taken to reduce the damage caused by this relationship.

Seed Infection Mechanisms [3]

The area of science that studies the relationship between pathogens and seeds is Seed Pathology. It does not only identify the pathogens, it also includes the role of the seed as source of inoculum, the survival of the pathogen and the actions taken to control the pathogens associated to it. It uses the knowledge of General Pathology, Microbiology and Seed Analysis.

To obtain a perspective of seed borne diseases, seed borne microorganisms can be considered fewer than four classes. The first consists of pathogens for which the seed is the main source of inoculum; when seed infection is controlled, the disease is controlled. An example would be lettuce mosaic virus. For these pathogens, the importance of seed-borne inoculum has long been recognized, and control practices have been developed. The second class consists of important pathogens in which the seed borne phase of the disease is of minor significance as a source of inoculum. Examples are those in which the crop residues in the field were the major source of inoculum. The third and largest group of seed borne microorganisms consists of those that have never been shown to cause disease as a result of their presence on seeds. The fourth class is a group of microorganisms that can infect the seed either in the field or in storage and reduce yield and seed quality. Examples of field fungi are *Diplodia*, *Fusarium*, *Cladosporium*, etc. The storage fungi *Aspergillus* and *Penicillium* can invade most types of seeds under high-moisture storage conditions [4].

The process of seed infection is influenced by the conditions under which the crop grows. Between the facts that influence in the process of infection are: the host and its genotype, the pathogen and its genotype and environmental facts. An infected seed will not always be the cause of the infection in the plant which originates, so, it is to say that the infection caused from an infected seed is an exception, not something to happen generally [5].

There are two situations, Systemic infection of the seed and Contamination or Infestation of the seed.

I. Systemic Infection of the Seed

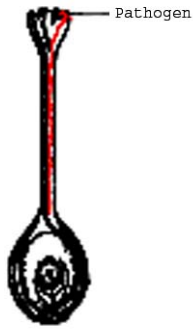
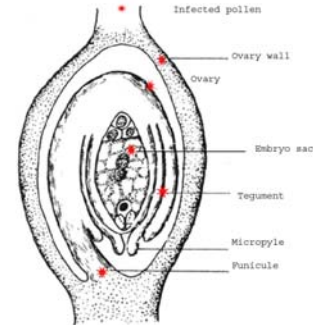
The establishment of a pathogen in any part of the seed is referred as seed infection. It can be systemic, by the vascular system or plasmodesmata or directly by natural or artificial wounds. The same pathogen can infect the seed using one or more of these mechanisms.



For example: *Xanthomonas campestris* pv. *phaseoli* can infect seed through the vascular system, by natural openings (From the pod suture goes to the funiculus, then to the raphe and tegument, or it can also happen through the micropyle).

A. Systemic infection through flowers, fruits or funiculus

Most of the systemic seed-borne bacteria and fungus reach and infect the embryo through the flower or from the peduncle of the fruit, via funiculus. Viruses go to the embryo from the systemically infected mother plant and the infected or contaminated pollen. They rarely reach the embryo during the formation of the seed or the embryo itself. Examples of some infections that occur through the vascular system are: Some *forma specialis* of *Fusarium oxysporum* in pumpkin, pea and tomato; *Plasmopara halstedii* in sunflower; *Septoria glycines* in soy; *Verticillium dahliae* in spinach and sugar beet; Certain pathovars of *Xanthomonas campestris* in bean, cabbage, rice and sweet pepper; and *Pseudomonas syringae* pv. *Lachymans* in cucumber.



B. Penetration through the stigma

During the infection, some pathogens follow the same path as the pollen grains do. Spores of some fungus reach the stigma and germinate, producing a hypha that reaches the ovary through the style, where they can stay as a dormant mycelium until seed germination. For example: *Ustilago nuda* and *U. tritici* in barley and wheat, and *Alternaria alternata* in sweet pepper. Viruses can infect through infected pollen, the male gamete carries the virus, when joining the ovule it generates an infected embryo. If both, the male and female gamete are infected they can even produce an infected endosperm.

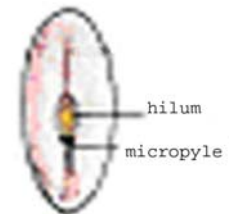
C. Penetration through the wall of the ovary or immature seed covers

Some fungi, like *Ustilago nuda* and *U. tritici* penetrate through the wall of the ovary as a result of the germination of the Teliospores on the stigma or the wall of the ovary. The pro-mycelium goes through the wall and other tissues until it reaches the embryo.

In some other cases, penetration occurs through breakages on the testa, establishing itself in the endopleura or the endosperm.

In fleshy fruits, like cucumber, melon, eggplant, tomato, sweet pepper and others, contamination can occur directly through the funiculus or in the tegument, during the process of seed formation. Examples of this are *Colletotrichum lagenarium* in watermelon and *Rhizoctonia solani*, when it invades fleshy fruits, like the ones mentioned above, it is capable of infecting from the placenta and penetrate to the developing ovule or seeds that are still in its formation process and have not lignified its cover.

D. Penetration through wounds and natural openings



Seedborne Pathogens

Natural openings like the hilum and the micropyle or wounds generated during the thresh are spots where pathogens like *Xanthomonas campestris* pv. *phaseolicola* in bean and *Pseudomonas syringae* pv. *lachrymans* in cucumber.

II. Seed contamination or infestation

Contamination or infestation refers to the passive relationship of a pathogens and seeds. The pathogen itself or parts of it can stick or can get mixed with the seeds during any of the processes during seed recollection: harvesting, extraction, thresh, selection and packing.

A. Pathogens that stick to the surface of the seed

Pathogens that stick to seeds during harvest or postharvest do so by their spores (Clamidospores, Oospores, Teliospores, Uredospores), bacterial cells and in some cases, virions.

Spores of the following fungi can be carried on seed coat surfaces:

Alternaria brassicae and *A. brassicicola* in crucifers; *A. longipes* in tobacco; *A. radicina* in carrot; *Ascochyta pinodella* in pea; *Drechslera sorokiniana* and *D. oryzae* in rice;

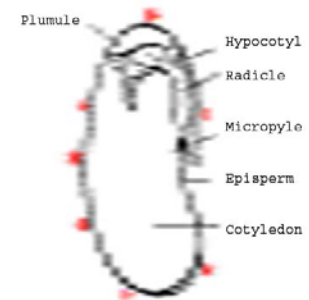
D. avenae in oats; *Fusarium oxysporum* f. sp. *callistephi* in China aster; Sclerotia of *Rhizoctonia solani* in eggplant, pepper, and tomato; *Tilletia caries*, *T. foetida*, *T. contraversa*, and *Urocystis agropyri* in wheat.

A number of bacteria, such as the following, contaminate seed surfaces:

Corynebacterium flaccumfaciens pv. *flaccumfaciens* in bean, *Pseudomonas syringae* pv. *phaseolicola* in bean, *P. syringae* pv. *lachrymans* in cucumber, *P. syringae* pv. *tomato* in tomato, *C. michiganense* pv. *michiganense* in pepper,

Xanthomonas campestris pv. *campestris* in cabbage

Also some viruses as Tobacco mosaic virus, Tomato mosaic virus, Pepper Mosaic virus.



B. Accompanying contamination

This type of contamination refers to physical mixing of the seed with pathogen's propagation organs like the sclerotium, nematode's galls, contaminated plant parts or soil particles containing pathogens.

Structures of the pathogens.

Some fungi produce resistance structures named sclerotiums, that are made of compacted mycelium. Under certain conditions of temperature and humidity it can germinate, it is common that this occurs together with the seed, producing its infection, by direct penetration or by the spores generated by the fructification organs (Apothecium or Perithecium). These spores are carried by the wind and taken to susceptible tissues of the plant, generally, some parts of the flower. Sclerotiums have many shapes and during thresh can be easily included with the rest of the seed. Some examples of this are *Sclerotinia sclerotiorum* in horticultural, grain and flower crops, *Sclerotinia cepivorum* in garlic and *Claviceps purpurea* in barley.

Mix with infected plant parts.

Infected plant's parts and residues can carry fructifications or spores of fungi and bacteria. This situation is particularly common in cereal, forage and oily crop's seeds. Examples of this are *Septoria nodorum* in wheat, *Puccinia malvacearum* in hollyhock (malvarrosa), *Colletotrichum trifolii* in clover, *Erwinia carotovora* pv. *carotovora* in tobacco, *Pseudomonas syringae* pv. *phaseolicola* in bean and *Sclerotinia sclerotiorum* in sunflower, soy and peanut.

Soil

Seeds can be mixed with contaminated soil and, for example, carry micro-sclerotium of *Macrophomina phaseolina* in dirty seeds of kidney bean, *Verticillium oxysporum* f.sp. *phaseoli* in cotton seeds, *Plasmodiophora brassicae* in turnip and *Fusarium oxysporum* f. sp. *phaseoli* in bean.

Seed Transmission

We saw how the seed gets infected or infested. Now we will see how these seeds can produce an ill plant. This is to say, how the inoculum goes from the seed to the plant.

We call seed-borne pathogens only those that can produce an infection. Because they have to be distinguished from those that can associate to the seed but do not produce an infection, these ones are called pathogens not transmitted by seed. For example, the virus that causes curly top in sugar beet can be present in the perisperm but does not cause an infection in the plant.

In general terms, infection can be classified into systemic and non systemic. It is systemic when the pathogen introduces itself to the plant when the seed germinates, and develops with it. Non systemic infection occurs when there is a localized infection caused by the pathogen in the seedling at the stage of pre or post emergency, in this situation there are no systemic symptoms. [6]

A. Systemic transmission

This type of infection can be produced by pathogens that are carried with the seed in various parts, like the embryo, endosperm or episperm, or by contamination of the outer portion of this one.

1. Infection of the embryo

When the seed germinates, if the embryo is infected, the pathogen initiates its growth together with the plant. Symptoms can show up during different stages of development. In the case of *Ustilago tritici*, the mycelium grows together with the plant and expresses symptoms only at the flowering stage, which are that all the tissues of the spike, except the rachis, are replaced by spores. Some pathovores of *Xanthomonas campestris* that infect cabbage, bean or sweet pepper move between the cells of the host until they reach the vascular system and produce symptoms in leaves or stems. Most of seed-borne viruses persist inside the embryo. Its multiplication and movement accompanies the plant during its development, and there can be symptoms at any stage, from the formation of first leaves until flowering or fructification.

2. *Non embryo infection*

Infection of the episperm (testa and endopleura) occasionally conducts to a systemic infection. Some bacteria, like *Corynebacterium michiganense* pv. *michiganense* in tomato and *Xanthomonas campestris* pv. *campestris* in cabbage, penetrate through stomata of cotyledons, and from there, reach the vascular system, initiating the systemic infection.

3. *Episperm contamination*

In some few cases this type of contamination conducts to a systemic infection. Most of these exceptions are fungi that are highly specialized in their pathogenesis and produce in cereals the so called smuts, rusts or mildews. In these cases, generally, spores are carried outside the seed, they germinate, penetrate the coleoptile, and start a systemic infection. This can occur directly o by a more complex system of haploid hypha fusion in genera like *Tilletia*, *Ustilago*, *Uromyces*, *Sclerospora*, *Pernospora* and *Puccinia*.

B. Non systemic transmittion

Non systemic infection is very common, and in the same way as systemic infection, it can come from an infection, outside contamination or by pathogens mixed with the seeds.

1. *Infection of the embryo*

This case is restricted to some pathogenic fungi that maintain itself in the embryo or the episperm as hypha inside the seed. Primary infection starts as injuries in the cotyledons or primary leaves, stems or petiole. Fungi fructifications (Pycnidium, Acervulus) can develop on these organs, under certain favorable conditions (temperature and humidity). These fructifications produce spores that, with the action of water and wind, disperse the disease to other parts of the plant and other plants. This occurs in *Ascochyta pisi* in pea, *Colletotrichum lindemuthianum* in bean and *C. truncatum* in soy.

2. *Infection of the episperm*

Generally, seeds in which the episperm is infected, do not geminate, or germinate and contaminate the soil. Rarely produce a systemic infection, but can infect the seedling from the outside. Over the injuries, new inoculum is produced, this one infects other parts of the plant or other plants. For example, *Septoria nodorum* in wheat under high humidity conditions, forms Picnidium in the coleoptile, whose spores disseminate the disease to other plants.

3. *Contamination of the episperm*

Contamination outside the seed's testa can produce healthy seedlings, but the inoculum infects the soil and from there it can cause infections at more developed stages of the plant. Wheat and rice grains that are contaminated with Teliospores of *Neovossia indica* in wheat and *N. torrida* in rice, produce ESPORIDISPORAS in the soil, that can be carried by the wind and infect flowers, originating the smuts.

4. *Accompanying contamination*

The mixture of seeds with sclerotiums of the fungi or contaminated soil particles produces non systemic infections, at any stage of the plant growth and development. It is common for *Sclerotinia sclerotiorum* to produce mycelium from the sclerotiums. These mycelium can directly infect the

plant or produce fructifications that produce spores that can be carried by the wind and infect flowers of different species, like sunflower, soy, peanut, etc.

Prevention of Seedborne Diseases

Prevention of seed-borne diseases is based in two main facts. On one hand there is the usage of material free from pathogens and contaminants that could originate a disease and on the other there are the treatments to eliminate the inoculum in the seed or propagule.

When using material free from pathogens it is important to have adequate pathogen detection methods, this is to say, sensible and specific. No production of health quality seed can be done without valid methods to detect the possibility of contamination.

Some treatments can be necessary to eliminate the pathogens from the seeds, or at leaf, maintain an economically viable balance.

Health testing of the seed

One of the most relevant aspects about controlled health quality seed is referred to the detection methods used on every case. ISTA (International Seed Testing Association) appeared in 1924, by was only interested in seed's gene purity and germination aspects. Later then, thanks to the efforts made by Dr. L. C. Doyer publishing the first Manual for Determination of Seed Borne Disease and being the first director of the PDC (Plant Disease Committee), the importance of seed-borne diseases was turned unto first priority. But detection methods were applied according to the criterion of each laboratory, so their results were not comparative. So, in 1957, PDC established a comparative health testing program and standardize applied methods. The complexity for comparison of these methods made a considerable step back on the publication of working sheets (protocols). ISTA approved methods became Rules. By 1999 only 64 protocols were being compared, and only 14 of them were accepted as Rules. As the solutions proposed by ISTA did not satisfy the needs of international seed industry, this industry, in 1994, organized The International Seed Health Initiative for Vegetables (ISHI-Veg) chartered by the vegetable seed industries in The Netherlands and France. The Initiative was soon joined by the seed companies in the United States, Israel and Japan. This group represents the production of over 75% of the worlds vegetable seed supply [7].

ISHI published many protocols, organized by crop. For horticultural crops, the ISHI-VEG, the Seed Health Testing Methods Reference Manual [8], this one included 21 different protocols with diverse status ranking (see Table1).

ISHI-Veg status

1. An ISHI Validated Reference Method is a method that has been through the ISTA/ISHI comparative testing process and is being published as ISTA working sheet.
2. An ISHI Reference Method is a method that has been through ISHI comparative testing and is under review by the ISTA/ISHI reviewers for publication as ISTA Working Sheet.

3. An ISHI Accepted Method is a method that is commonly used by the seed industry for determining seed health. The method has been published in a journal, as a working sheet and/or is publicly available for comparative testing and commonly in use by the seed industry. The method is in comparative testing or aspects of the test are being tested for inclusion or deletion from the method.

4. An ISHI Reviewed Method is a method that is publicly available or published, documented and prioritized by the ITG, but not subjected to a comparative test at this time. The method usually is accepted by other agencies or groups.

In 1995, during the second ISTA-PDC symposium it was decided that a Joint ISTA/ISHI Guidelines for Comparative Testing of Methods for Detection of Seed Borne Pathogens would be edited. Through this combined effort many laboratories, organized under this alignments, the ISTA developed the Manual of Seed Health Testing Methods, that has two sections: I.- Validated Test Methods and II.- Peer Reviewed Methods [9].

The transition process from working protocol to ruled method is long and laborious, so working protocols can be considered as adequate methods for seed analysis. Nowadays, it is common that technological advances may become obsolete even before they are published. For example, the protocol to analyze *Xanthomonas campestris* pv. *campestris*, which causes black rot in crucifers, was published in 1982, there, it was recommended one minute to extract the bacteria from the seeds to starch culture medium. 2 years later, this time was raised to 2 hours, and other specific culture mediums. In 1987 the protocol was replaced, again in 1996 and at present time again it is under revision.

New techniques for seed health analysis

Pathogen detection methods have suffered great changes since 1957. Back then it was mainly targeted to fungi, and based exclusively on incubation and identification, or the cultivation of these seeds and counting the percentage of unhealthy seedlings. These analyses were very laborious and required a high infection level in order to be detected. Nowadays, technological advances have allowed detecting even low infection levels, evaluating 1,000 to 10,000 seeds at once. Specificity of culture mediums, usage of antibiotics and other products has allowed isolating the pathogen from the seed. Together with this, the use of reactivities as mono or polyclonal antisera and molecular markers is also available. For example, for the Lettuce Mosaic Virus (LMV), back in 1950 the symptoms were observed over many thousands of seedlings from the seed that were going to be analyzed. Later, technical advances determined that 30,000 seedlings were needed, and even later, in 1983, an alternative to the *Chenopodium quinoa* test was discovered, the ELISA test.

Watermelon Fruit Blotch caused by a bacteria is a severe problem in glasshouse winter production. It was determined that a single contaminated seed in 9,000 of them was enough to widespread the bacteria to the rest of the plants. That is the reason why screening was done over 10,000 seedlings. Nowadays PCR technique is used [9].

Minimum requirements to produce basic and certified seed

The Organization for Economic Cooperation and Development (OECD) establishes steps to be taken in order to produce basic and certified seed. Between these steps it is emphasized that generations from the mother material to the basic material are strictly limited in number. The number of certified seed generations, after the basic seed, varies from crop to crop and technical conditions [10].

In both, sexually and vegetative reproduced species, the schemes include similar steps. It generally starts with selection of a plant with genetic and health advantages. If health condition was not satisfying, certain actions should be taken in order to release it from considered pathogens. This Mother Plant is maintained under strict special security conditions in order to maintain healthiness (isolated glasshouses or chambers).

In the Certification Schemes two particularities about seed borne pathogens should be considered, those transmitted exclusively with the seed and those that can also be transmitted with contaminated soil and plant rests. Diseases transmitted exclusively with the seed can be controlled by using clean healthy seed. This seed can be obtained by Seed Certification Programs or by treatments that are really effective, like *in vitro* culture, thermotherapy, chemotherapy or a combined action of these, for systemic pathogens.

Tolerance levels in certified seed depend on the multiplication speed and dispersion of the inoculum of the pathogen of the considered disease. If a small amount of inoculum may be epidemic, like Anthracnose and the bean bacterial blight, if levels above zero chemical control may not be enough to stop the disease. But pathogens whose inoculum multiplies slowly generation after generation, the disease may be evident only several generations later, so, if contamination levels are above zero may be accepted in adequate control methods are applied to the seed. This is what happens in wheat, with the Loose Smut, continuous treatments diminish inoculum levels, and make it perfectly tolerable.

In the OECD [10] scheme it is considered:

1.- Health of Seed Used for Crop Production

The seed used for seed crop production should be as pest and disease free as possible. Its health should be checked before use and, if pest or disease organisms against which there is an effective seed treatment are present, that treatment should be applied.

2.- Previous cropping

- 2.1 Seed production fields or glasshouses shall be sufficiently free from volunteer plants to avoid contamination of the crop seed by:
 - 2.1.1 Any seed which is difficult to remove from the crop seed
 - 2.1.2 Cross-pollination;
 - 2.1.3 Seed-borne diseases transmitted from volunteer plants
- 2.2 The previous cropping shall be such that there is the least possible risk of any soil borne diseases being present which could subsequently be transmitted in the harvested seed.

2.3 If any previous crop could have made the field or greenhouses unsuitable for the above reasons, adequate measures must be taken.

3.- Isolation

3.1 Seed crops shall be isolated from all sources of pollen contamination and seed-borne diseases (including seed-borne virus infection and wild plants that might serve as a source of disease). In particular, the distances must not be less than those showed in Table 2.

3.2 The distances apply both to other seed crops and to plants or crops grown for vegetable production flowering at the same time as the seed crop. They can be disregarded when there is sufficient protection from undesirable pollen sources and seed-borne diseases (e.g. crops produced in aphid-proof glasshouses).

4.- Field Inspection

4.1. Each crop of Basic Seed shall be inspected at least once at an appropriate stage of growth on behalf of the Designated Authority by inspectors who are specially trained and, in their inspections, responsible only to the Designated Authority.

4.2. At least 20 per cent of the crop of Certified Seed of each species shall be inspected by these inspectors.

4.3. Each crop of Certified Seed shall be inspected under the responsibility of the person responsible for the production of Certified Seed

4.4. The field inspector shall check that all the minimum requirements laid down in this Appendix have been satisfied.

4.5. The crop must be satisfactory as regard to varieties identity and purity.

4.6. The presence of any seed-borne diseases shall be at the lowest possible level.

References

1. Font Quer, P., Diccionario de Botánica. 1982, Barcelona, España: Editorial Labor, S. A. 1244.
2. Hartmann, H.T. and D.E. Kester, Propagación de Plantas. 6 ed. 1971, Mexico: Compañía Editorial Continental, S. A., Mexico. 810.
3. Agarwal, V.K. and J.B. Sinclair, Mechanism of seed infection, in Principles of seed pathology. 1987, CRC Press, Inc. p. 176.
4. McGee, D.C., Seed Pathology: Its place in modern Seed Production. Plant Disease, 1981. 65(8): p. 638-642.
5. Reis, E., Melo, D. Barreto, and M. Carmona, Patología de semillas en cereales de invierno. 1 ed. 1999, Buenos Aires, Argentina: Gráfica Condal S. R. L. 94.
6. Agarwal, V.K. and J.B. Sinclair, Seed transmission and inoculation, in Principles of seed pathology. 1987, CRC Press, Inc. p. 176.
7. Sheppard, J., Importance of seed health within seed testing. 1999, International Seed Testing Association-ISTA: Basserdorf, CH-Switzerland. p. 1-8.

8. van Ettekoven, K., SeedHealth Testing Methods Reference Manual. 2002, ISHI-VEG, International Seed Health Initiative for Vegetable Seeds.
9. Sheppard, J. and H. Wesseling, Joint ISTA/ISHI Guidelines Joint ISTA/ISHI Guidelines for Comparative Testing of Methods for Detection of Methods for Detection of Seed-borne Seed-borne Pathogens. 1997, ISTA-ISHI. p. 17.
10. OECD, RULES AND DIRECTIONS OF THE OECD SEED SCHEMES: Vegetables, in OECD Schemes for the Varietal Certification or the Control of Seed Moving in International Trade, O.C.D. C(2000)146/FINAL, Editor. 2000. p. 204-205.

Table 1. Seed Health Testing Methods Reference Manual, protocols and acceptance levels.

Nr	Crop	Pathogen	Status
1	Bean	<i>Colletotrichum lindemuthianum</i>	3
2	Bean	<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i>	3
3	Bean	<i>Xanthomonas campestris</i> pv. <i>phaseoli</i>	3
4	Beet	<i>Phoma betae</i>	3
5	Brassicaceae	<i>Phoma lingam</i>	4
6	Brassicaceae	<i>Xanthomonas campestris</i>	2
7	Broad bean	<i>Ditylenchus dipsaci</i>	4
8	Carrot	<i>Alternaria dauci</i>	3
9	Carrot	<i>Alternaria radicina</i>	2
10	Carrot	<i>Xanthomonas campestris</i> pv. <i>carotae</i>	3
11	Corn salad	<i>Peronospora valerianellae</i>	4
12	Cucurbitaceae	<i>Acidovorax avenae</i> spp. <i>citrulli</i>	3
13	Cucurbitaceae	<i>Didymella bryoniae</i>	3
14	Lettuce	<i>Lettuce Mosaic Virus</i>	2
15	Pea	<i>Ascochyta pisi</i>	3
16	Pepper	<i>Tobamovirus</i>	3
17	Pepper	<i>Xanthomonas campestris</i>	3
18	Tomato	<i>Clavibacter michiganensis</i>	3
19	Tomato	<i>Fusarium oxysporum</i>	4
20	Tomato	<i>Xanthomonas campestris</i>	3
21	Tomato	<i>Tobamovirus</i>	3

Table 2. Minimum isolation distances from all sources of pollen contamination and seed-borne diseases.

		Minimum distances	
		Basic Seed	Certified Seed
1	When the foreign pollen can cause serious deterioration in varieties of Beta and Brassica species	1000 m	600 m
2	From other sources of foreign pollen affecting varieties of Beta and Brassica species	500 m	300 m
3	When the foreign pollen can cause serious deterioration in varieties of all other cross-pollinating species	500 m	300 m
4	From other sources of foreign pollen affecting varieties of all other cross-pollinating species	300 m	100 m