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Asia and Pacific Plant Protection Commission

REGIONAL IMPLEMENTATION GUIDANCE

Phytosanitary procedures for seed certification

APPPC RIG No. 1

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Food and Agriculture Organization of the United Nations
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INTRODUCTION

Scope

This Regional Implementation Guidance provides overarching guidance for the Asia and Pacific Plant Protection Commission (APPPC) member countries on how to implement International Standard for Phytosanitary Measures (ISPM) 38 (*International movement of seeds*) when producing seeds for planting. It describes practical phytosanitary measures that can reduce pest risks in each step of the seed export system and should be read in conjunction with ISPM 38.

The appendix to this guidance includes descriptions and information for capsicum seed, with examples of pests that may be associated with the commodity, and examples of potential seed treatments, diagnostic testing protocols and other pest detection methods that may be acceptable measures. More appendices may be added for other seed commodities in the future.

This guidance does not address seed quality.

Background

International seed trade around the world grows significantly every year. Asia and the Pacific region is one of the key markets for seed and is home to some of the world's major seed-producing countries. Importing seeds for planting is associated with risks of seedborne, seed-transmitted, and contaminating pests entering a country, becoming established and spreading when infested¹ seeds are introduced to an environment that is favourable to the pest. Therefore, phytosanitary measures are often necessary. Measures should be harmonized among the member countries as much as possible. This will optimize how pest risks resulting from the movement of seeds in the region are managed, noting that the status of pests and appropriate levels of protection can vary from country to country.

ISPM 38 was adopted by the Twelfth Session of the Commission on Phytosanitary Measures in 2017. It provides guidance that national plant protection organizations (NPPOs) should follow when identifying, assessing and managing the pest risks associated with the international movement of seeds.

¹ The term *infest / infested* is used throughout this document for consistency with *Explanatory document on ISPM 5 (Glossary of Phytosanitary Terms)* revised in 2022

PHYTOSANITARY PROCEDURES FOR SEED CERTIFICATION

1. General information

1.1 Commercial seed production process

New varieties of agronomic and vegetable crops are commercially grown from seeds. Depending on the crop, new varieties may be open-pollinated or hybrid. Plant breeders develop open-pollinated varieties by selecting parent plants with desirable characteristics, such as high yield or disease resistance, and breeding from those plants. After several breeding cycles, genetically stable offspring with desirable traits are selected and then commercialized as a new variety. Open-pollinated varieties breed true, meaning they retain the same characteristics when multiplied. Most seeds moved in international trade of open-pollinated crops, such as ryegrass, clover and peas, are not usually parental lines, but are established commercial varieties.

Other crops such as tomato, capsicum and corn are commercially grown from hybrid seeds. The quality of the seed used to produce these crops depends heavily on the starting material or parental seeds. The parental seeds are two genetically different lines with desirable traits developed by plant breeders. The parental lines are crossed to produce a new improved hybrid variety that is commercialized. Hybrid varieties may contain genes that confer traits such as disease resistance, drought-tolerance, high yield or nutrition-enhancement in progeny plants.

The first step in the commercial hybrid seed production process is to sow the parental seeds intensively and multiply them under conditions that ensure they retain their genetic purity. The purpose of multiplying parental seeds is to produce enough starting material (male and female seeds) for commercial hybrid seed production. The parental seed lines are then sowed to produce hybrid seeds.

Seeds for planting that are harvested from crops, whether open-pollinated or hybrid varieties, are cleaned, dried, treated and packaged before they reach their final destination. Seeds are dried soon after harvest to reduce their moisture content according to the seed specification of each crop. This gives the seeds their maximum shelf life. The seeds may be graded based on size and treated with fungicides or other additives (APSA and ISF, 2017).

1.2 Global seed trade aspects

Asia and the Pacific is the region with the fastest-growing seed market and is home to some of the world's major seed-producing countries. In 2018, more than USD 4.1 billion worth of sowing seeds were traded in the region, constituting about 14 percent of the global seed trade (APSA, 2022).

Most trade of commodities is based on a simple model where a product is produced in one country and exported to another. The global seed trade is complex and involves seeds moving between and through various locations worldwide. Seeds are moved internationally for sale, production, trialling, bulking-up or multiplication, breeding, and other purposes. As seeds move through the stages from breeder seed to commercial production, it is not uncommon for seeds to pass through multiple countries before they end up with the final consumer (Figure 1). Many countries in Asia and the Pacific region are involved in these stages of seed production.

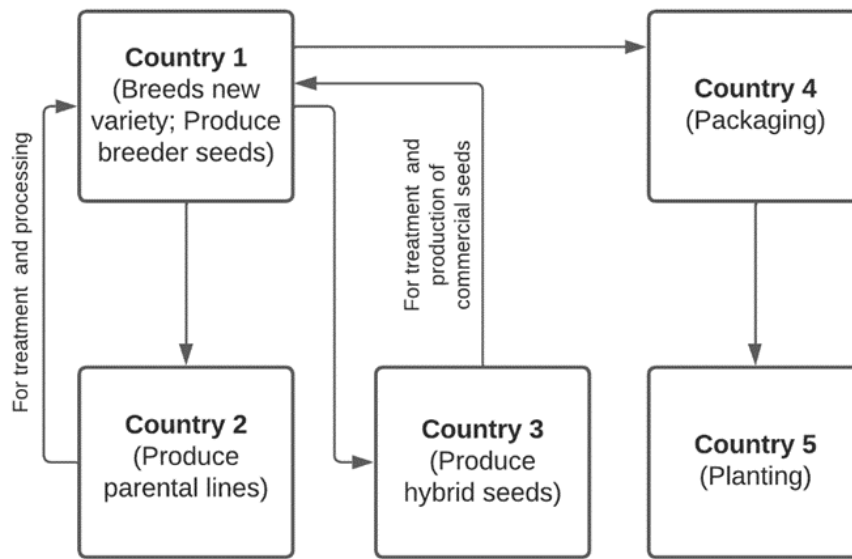


Figure 1: Example of the movement of seeds in global trade

1.3 Pest risks associated with seeds

Seeds for planting can present a pest risk as pests associated with seeds can become established, spread in the environment and cause unwanted impacts. This is because seeds that are planted are intentionally introduced into a place of production and a production area. If a seed is infested with pests, the pest may be introduced to other countries and regions through global trade.

As the seed trade grows, the geographic ranges of seedborne pathogens are expanding, and new risks frequently emerge. Asia and the Pacific region is no exception. For example, *Tomato brown rugose fruit virus* (ToBRFV) is a newly identified virus affecting the production of tomato (*Solanum lycopersicum*) and capsicum (*Capsicum annuum*). ToBRFV was first recorded in the Jordan Valley and Israel between the autumn of 2014 and the spring of 2015 but has now invaded other tomato-growing areas in the world (France, Germany, Greece, Italy, Mexico, Netherlands, Spain, the United Kingdom of Great Britain and Northern Ireland, and the United States of America (California)). Some spread has been attributed to local trade of infested seeds and seedlings (CABI, 2022). However, spread between larger regions (i.e. continents) can be attributed to seeds moving internationally. ToBRFV has been intercepted even in Asia and the Pacific region from tomato and pepper seeds imported from the outbreak countries (PPS of Japan, 2021). The risk of the virus being introduced to this region through the seed trade has been increasing.

When pests are introduced by seeds into previously uninfested areas, the absence of suitable control measures can lead to severe yield and quality losses. Losses are not always restricted to the fields where diseased seeds are sown. Secondary inoculum can be carried by wind, rain, irrigation water, machinery, insects, animals and humans spreading the pests long distances from the original infestation. Ensuring that seeds are healthy is a precondition for healthy crops and is crucial for food security and sustainability, not only for Asia and the Pacific but for regions that import seeds from Asia and the Pacific region as well.

1.4 The role of pest risk analysis in phytosanitary certification of seeds

Seeds are a pathway by which a wide range of pests can be introduced to a country and spread. Seeds can bring with them fungi, bacteria, viruses, viroids, nematodes, weeds, and arthropods. The significance of seeds as a pathway for pest introduction depends on the specific host–pest combination. Pests with a wide host range may be seed-transmitted in some hosts but not in other hosts. Host seeds that are not seed-transmitters of a pest do not act as a direct pathway for introduction of that pest, however, may act as an indirect pathway for introduction.

Under the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement), each country has the sovereign authority to use phytosanitary measures to regulate the entry of seeds that can harbour plant pests. However, phytosanitary import requirements need to be technically justified. A pest risk analysis (PRA) provides the basis for technical justification for regulating pests on seeds. Importing countries should conduct PRA for seeds in accordance with ISPM 2 (*Framework for pest risk analysis*), ISPM 11 (*Pest risk analysis for quarantine pests*), and ISPM 21 (*Pest risk analysis for regulated non-quarantine pests*) when setting their phytosanitary import requirements.

A PRA for seeds imported for the purpose of planting should identify regulated pests potentially associated with the seeds. The categories of pests that may be potentially associated with seeds include seedborne pests, and contaminating pests such as arthropods, fungal structures or seeds of plants as pests (i.e. weed seeds). A pest is seedborne when it is carried externally or internally by seeds. A pest is seed-transmitted when it is both seedborne and transmitted directly from the seeds to the plants that grow from the seeds. By contrast, a seedborne pest that is not seed-transmitted may still infest plants in the field by transferring to the environment and then infesting a plant under natural conditions, for example through the soil or water, or via a vector. When assessing seedborne pests, the ability of the pest to transfer from the seed to a suitable host to cause infestation needs to be considered to identify the pests that justify regulation. Seedborne pests that do not transfer to a host plant under natural conditions should not be regulated. The PRA should assess the probability of the pest becoming established, spreading and causing economic impacts in the area. If the risk from a pest is unacceptable, countries may require phytosanitary measures to manage the risk on imported seeds. The PRA should consider risk management options for regulated pests and recommend measures that are proportional to the risk and are least restrictive to trade.

The mechanisms of transmission and infestation of seedborne pests can vary by pest species or host plants. The management of seedborne pests should be underpinned by a thorough knowledge of the pest, host crop and its production system, the environment and their interactions, and the precise identification of the pests. Information gathering and analysis are important. Suitable management strategies can be devised and tested as more information becomes available.

When the NPPO is assessing pest risk and developing pest risk management options for a specific pest associated with host seeds for planting, assessors should consider:

- the life cycle of the pest and the host;
- pest epidemiology;
- potential sources of pest infestation and contamination (shown in Table 1) and the mechanism by which parent plants used for seed production could become infested with a seedborne pest or a seed lot could become infested with a contaminating pest; and
- the impact of production practices on managing pest risk.

The NPPO of the seed-exporting country should develop phytosanitary procedures and take appropriate actions to the extent necessary to meet the phytosanitary requirements of the importing country. A procedure for phytosanitary certification in the seed export system could involve various potential pest risk management practices applied at any stage along the seed production process (e.g. before planting, before harvest, at harvest, and during post-harvest handling) and evaluated as appropriately reducing the pest risk. Each action could be applied alone (e.g. a seed test) or in combination with another (e.g. in the case of a systems approach) to manage pest risk and meet the phytosanitary import requirements and reduce the risk of seedborne pests spreading through the global seed trade.

ISPM 38 provides guidance to help NPPOs to identify, assess, and manage the pest risks associated with the international movement of seeds.

Table 1: Potential sources of pest infestation and contamination of seeds produced for field planting

Potential sources of pest infestation and contamination	Explanation
Seeds	Seeds may carry seedborne pests or contaminating pests. The association of pests with seeds can be categorized according to the location of the pests: 1) presence in the seed embryo, 2) presence in seed parts other than the embryo, and 3) external contamination of seeds. As seed trading has become global, diseases can be transmitted over long distances through infested seeds.
Air	Airborne pests such as fungal spores can spread by wind and air currents. Arthropod pests and vectors such as insects and mites can be spread by wind or their own flight.
Water	Waterborne pests can spread to a crop directly via rain splash and irrigation water or indirectly through increased humidity. Greenhouse plants could be exposed to inoculum by using recycled water for irrigation.
Fungi	Some pests (for example certain viruses) are vectored by fungi. These fungi mostly inhabit soil.
Soil	Some bacteria, oomycetes, fungi, viruses and nematodes may persist in the soil for years as microspores, oospores, cysts, etc. and can serve as a source of inoculum to infest plants in the next cropping season.
Crop/plant residues	The decaying parts of the crop plant that are not harvested and are sometimes considered waste can harbour bacterial and fungal pests that survive in the crop residues. These pests can become a source of inoculum in the next cropping season. Soil mixed with infested crop residues can also serve as a source of infestation.
Arthropods	Some arthropods actively transmit plant viruses and bacterial pests. Some arthropods in the field feed on and in seeds during the seed development period, before

Potential sources of pest infestation and contamination	Explanation
	harvest. Stored product arthropods can infest seeds after harvest.
Nematodes	Some species of nematodes are plant virus vectors. They mostly inhabit soil as internal or external root parasites. Some nematodes attack seeds, typically infesting seed lots as galls that form in place of seeds on infested mother plants. Some nematodes can be external contaminants if the seeds are not thoroughly cleaned (e.g. cyst nematodes, peds).
Hands, clothes and equipment	Some viruses, viroids and bacteria are mechanically transmitted. Plant wounds facilitate infestation because the wounds create points of entry and increase the likelihood of other pests infesting the plants.
Pollen	Some viruses and bacteria can spread via infested pollen.
Weed seeds	Some weeds may not show obvious symptoms of disease but still serve as reservoirs for viruses and bacteria that can be transmitted to other plant species. Weed seeds may be found as contaminants in seed lots.
Adjoining fields	Adjoining fields can be a source of infestation either by serving as a reservoir for plant pests and diseases or by discharging drainage water that could be infested by soilborne diseases.

Sources:

APSA (Asia and Pacific Seed Association) & ISF (International Seed Federation). 2020. *Good Practices for Healthy Vegetable Seed Production*. [Cited 8 June 2022]. https://www.apsaseed.org/storage/2020/10/APSA%20ISF%20Good%20Practices%20for%20Healthy%20Vegetable%20Seed%20Production_88436.pdf

CABI Compendium. 2022. [Cited 8 June 2022]. <https://www.cabidigitallibrary.org/journal/cabicompendium>

ISPM 38. 2017. *International movement of seeds*. Rome, IPPC Secretariat, FAO.

Sheppard, J.W. 1998. Seed-borne Pathogens of Vegetable and Flower Seeds: Their Devastation, Identification and Control. *Seed Technology*, Vol. 20, No. 2, Special Publication - A Symposium: Vegetable and Flower Seed Quality (1998), pp. 187-197.

USDA/APHIS (United States Department of Agriculture/Animal and Plant Health Inspection Service). 2019. *A Regulatory Framework for Seed Health (ReFreSH)*. [Cited 8 June 2022]. https://www.aphis.usda.gov/plant_health/downloads/refresh/refresh-concept-paper.pdf

1.5 Communication

The successful implementation of phytosanitary measures is dependent on good communication between importing and exporting NPPOs and industry participants. Examples of when NPPOs can communicate include:

- for the exchange of information for the development of the PRA;
- consulting on lists of pests and proposed phytosanitary measures; and
- notifying trading partners of phytosanitary measures including transition periods for implementation of new requirements (e.g. through the World Trade Organization (WTO) Technical Barriers to Trade (TBT) notifications platform).

2. Phytosanitary measures

2.1 Purpose of phytosanitary measures

Phytosanitary measures can be applied for a number of reasons including but not limited to:

- ensure that the area or place of production that the seed is planted is free from a pest;
- prevent the seed becoming infested during production;
- reduce infestation in the seed to a low-level during production;
- disinfest a consignment before export; and
- verify a consignment is pest free.

2.2 Assessing and choosing phytosanitary measures

Phytosanitary measures should be proportional to the risk posed by the pest, as determined by pest risk assessment, and the measure should be feasible to apply. When an NPPO is assessing the feasibility of a measure, assessors should consider these factors:

- the effect of the measure on the commodity (e.g. phytotoxicity) and environment;
- cost-effectiveness;
- availability of facilities, equipment or technology to implement the measure;
- authorization of the measure for use (e.g. registration of a chemical); and
- operational and technical practicality (e.g. timing of the application of the measure).

The type of phytosanitary measure and when it is applied will depend on the biological characteristics of the pest or pest group the measure aims to manage.

2.3 Options for phytosanitary measures

Options for phytosanitary measures for seed include:

- pest-free areas, pest-free places of production, pest-free production sites;
- areas of low pest prevalence;
- systems approach;
- in-field sanitation, crop treatment and field inspection during seed production;
- seed cleaning;
- seed treatment;
- inspection of seed consignments;
- testing of seed consignments for pest freedom for phytosanitary certification; and
- post-entry quarantine.

Each sub-section below describes options for phytosanitary measures, factors that need to be considered for implementation, and activities that support implementation of the measures.

2.3.1 Pest-free area (PFA), pest-free place of production (PFPP) and pest-free production site (PFPS)

The concept of ‘pest freedom’ for a regulated pest can be applied to an officially defined country, part of a country or all or parts of several countries. Recognition of pest freedom in these areas by a seed-producing country and an importing country can be a risk management option for phytosanitary certification of seeds to meet the importing country’s requirements.

The area may be:

- a whole country or an area within a country (PFA);
- a place of production (PFPP); or
- a production site (PFPS).

The suitability of a ‘pest freedom’ option depends primarily on the pest status in the targeted area, i.e. whether the pest is present (area infested) or absent (area not infested), and the objective of the pest control programme. The NPPO of the seed-producing country should assess the pest status in a targeted area according to the guidelines in ISPM 8 (*Determination of pest status in an area*).

If a pest is present, the NPPO should consider the rate of natural spread of the pest when deciding on the general suitability of pest-free measures. For example, PFA may be suitable for a pest with a high range of natural spread (e.g. > 10 km), PFPP for a pest with a low range of natural spread (e.g. > 10 m but < 10 km) and PFPS for a pest with a very low range of natural spread (< 10 m). PFPS or PFPP may be suitable options where plants can be physically contained such as production in a greenhouse.

PFA, PFPP and PFPS are required to be implemented as very structured programmes under the responsibility of the NPPO of the seed-producing country. A PFA is managed as a whole by the NPPO of the exporting country. PFA, when used alone as a phytosanitary measure, is sufficient for managing pest risk. A PFPP or PFPS is managed individually by the producer, under the supervision and responsibility of the NPPO.

NPPOs should follow the requirements for determining, establishing, maintaining, verifying, and recognizing pest freedom options that are described in ISPM 4 (*Requirements for the establishment of pest free areas*) and ISPM 10 (*Requirements for the establishment of pest free places of production and pest free production sites*). PFAs, PFPPs and PFPSs should meet the requirements in the relevant ISPMs.

Guidance on implementing these measures is described in the International Plant Protection Convention’s (IPPC’s) *Guide for establishing and maintaining pest free areas* (2019).

2.3.2 Area of low pest prevalence (ALPP)

An ALPP is a pest management option used to maintain or reduce the population of a pest regulated by an importing country below a certain threshold in a specific area. An ALPP can be used to facilitate the export of seeds for planting out of a country or part of a country where a regulated pest is present at a low level and which is subject to effective surveillance or control measures.

NPPOs should follow the requirements and procedures for establishing, maintaining and verifying ALPP in ISPM 22 (*Requirements for the establishment of areas of low pest prevalence*). The specific phytosanitary measures to be applied by the exporting country to establish, maintain and verify ALPP should be specified in an official operational plan under an arrangement between the NPPOs of the importing and exporting countries, or be a general requirement of the importing country.

The procedure for the recognition of ALPP is described in ISPM 29 (*Recognition of pest free areas and areas of low pest prevalence*). For guidance on implementing an ALPP, see the IPPC’s *Guide for establishing and maintaining pest free areas* (2019).

2.3.3 Systems approach

A systems approach is one of the options that a country exporting seeds could use to meet the phytosanitary requirements of an importing country that recognizes the exporting country's system as effective at reducing the pest risk to an acceptable level. A systems approach for seed will likely require multilateral discussions and arrangements due to the nature of seed production and supply system, which can involve multiple countries.

A systems approach integrates different measures for pest risk management, at least two of which act independently, in order to meet a predetermined level of protection with cumulative effect. Pest management practices and quality systems used by the seed industry during the pre-planting, pre-harvest, harvest and post-harvest phases of seed production, such as the measures described in Tables 2, 3, 4 and 5 in this guidance, may be integrated in a systems approach.

The NPPO should select measures based on the pest risk of the seed commodity and its production and supply system. A pest freedom measure such as pest-free places of production may be used as an independent measure at the pre-planting, pre-harvest, harvest or post-harvest phases of seed production or when it is not feasible to maintain pest freedom throughout the production cycle.

NPPO of importing countries may evaluate and authorize a systems approach as effective at reducing pest risk to an acceptable level and deem it an equivalent alternative to phytosanitary certification of seeds based on testing and inspection at the time of issuing a phytosanitary certificate. While the need to develop a systems approach as a phytosanitary measure for a seed commodity may be initiated by the seed industry, NPPOs of exporting countries are responsible for evaluating the effectiveness of the pest management practices used in a systems approach for reducing pest risk as well as responsible for auditing and monitoring the system to check that the effectiveness is maintained.

The guidelines for the development and evaluation of integrated measures in a systems approach as an option for pest risk management are described in ISPM 14 (*The use of integrated measures in a systems approach for pest risk management*).

Good Seed and Plant Practices (GSPP) is an example of a certification scheme whose purpose is to prevent tomato seed and plants from being infested by *Clavibacter michiganensis* spp. *michiganensis* (*Cmm*). GSPP integrates independent measures for managing pest risk from *Cmm* in the production chain in a manner described in ISPM 14. Seeds used as starting material for seed production are tested to confirm they are free from *Cmm* and hygiene protocols are followed during production to prevent infestation of the crop with *Cmm*. The general principles of the system are laid out in the GSPP Standard. Accredited companies that fulfil all the requirements of the GSPP standard ensure the availability of healthy tomato seeds and plants free from *Cmm*. The requirements include a quality management system, risk assessment of defined threats (water, people, propagation material and materials) in production processes and its control measures, and technical requirements. A further description of the GSPP scheme can be found in Peusens *et al.* (2020).

2.3.4 In-field sanitation, crop treatment, field inspection and other options

Phytosanitary measures can be integrated into the practices employed in seed production from the pre-planting phase through pre-harvest, harvest, post-harvest and distribution and transport phases until the seed reaches its import destination. Options can include in-field sanitation to control weeds that may act as reservoirs for pests or vectors of pests; crop treatments such as

chemical or biological pesticides to prevent or remove pests and; field inspection to monitor for symptoms of disease and trigger appropriate mitigation actions.

2.3.5 Seed cleaning

Seed cleaning is a mechanical process that can be applied after seeds are harvested and for some seed types, after they are dried. Cleaning or screening machines separate different sizes and weights of seeds through a system of sieves. The sieve system separates wanted seed from unwanted material such as debris that can harbour contaminating insects or fungal pests as well as weed seeds. Using sieving alone is likely to be inadequate where the wanted seed is of similar size, weight and shape as the unwanted seeds or material such as debris.

2.3.6 Seed treatment

Seed treatments involve exposing seeds to physical, chemical, or biological agents to reduce pest incidence on the seeds or prevent pests infesting seeds (seed protectants), particularly during seed storage, seed germination or early growth of the plant (e.g. seeds are treated with fungicide to prevent fungal infestation when the seed sprouts).

Seed treatment technology has undergone rapid changes. At the same time, the seed industry has developed new concepts, methods, materials, machines, growing structures, and cultural practices. Seed treatment is never an independent technology. It involves a series of procedures from seed harvesting to sowing. Treatment technology in horticultural crop seeds can be extremely variable and expensive, and the germination behaviour becomes more complicated due to the extreme diversity of crops, species, and cultivars (Lee, 2004). These treatments are used to protect seeds from soilborne pest infestation in the field and reduce or eliminate seedborne pest infestation with no effect on seed germination.

Where the importing country has specified seed treatment requirements (e.g. chemical to be used, dosage and treatment rate), the exporting NPPO should verify that the treatment has been conducted to meet these requirements. Treatment specifications/details can be included on the phytosanitary certificate. If an importing country has not specified a treatment, then it is the responsibility of the exporting NPPO to select an appropriate treatment. The treatment should be specific to the crop and pathogen, effective in eliminating the pest, rather than just suppressing it, and be supported by efficacy data.

2.3.7 Inspection of seed consignments

Inspection and/or testing to determine the presence or absence of regulated pests and regulated articles are usually conducted on representative samples of a seed lot or consignment. A seed consignment may comprise more than one seed lot, and a seed lot may be exported in more than one seed consignment over time.

In ISPM 5 (*Glossary of phytosanitary terms*), 'lot' is defined as "a number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment". The term 'seed lot' is used multiple times in ISPM 38 (*International movement of seeds*). According to ISPM 38, seeds of the same variety from different fields can be bulked immediately after harvest into a single lot; different seed lots of the same variety can be blended into a single lot; and seeds of different species, varieties or cultivars can be mixed into a single lot. While ISPM 38 does not mention the homogeneity of seeds that are bulked, blended or mixed into a single lot, it states that all seeds in a mixture, a blend or a bulk lot should meet the relevant phytosanitary import requirements. It also states that in assessing the pest risk of mixed, blended or bulked seeds, all combinations of pests, hosts and origins should be

considered, and the impacts of the mixing, blending or bulking processes should also be considered in determining the overall pest risk of mixtures, blends and bulk lots of seeds.

Regardless of whether a seed lot has been bulked, blended or mixed, the homogeneity of the seed lot is an important characteristic of the seed lot that needs to be considered in seed sampling.

2.3.8 Seed testing

Seed health testing using diagnostic methods such as molecular or serological methods can be used to detect and identify pests. Selected diagnostic methods (e.g. molecular and serological tests) should be validated to ensure sensitivity, specificity and reproducibility for detecting regulated pests in the sample. Diagnostic methods validated on other plant tissues may not necessarily be effective for seeds and therefore validation of the methods on specific seed species should be conducted.

2.3.9 Post-entry quarantine

The NPPO of an importing country may require post-entry quarantine for seeds as a measure in cases where seedborne pests are difficult to detect on or in seeds, symptom expression takes time, no seed tests or treatments are available for the pest.

The NPPO of the importing country may conduct post-entry quarantine to verify that imported parental seed lots are free from seedborne/seed-transmitted pests before the seeds are released into the field for seed production. As part of post-entry quarantine, a representative sample of the seed lot may be planted and the plants growing from the seeds tested for the presence of pests. Whether or not to implement post-entry quarantine on seeds is determined through pest risk analysis of seedborne pests associated with the seeds.

Where the imported seeds are associated with vector-transmitted pests (e.g. pea seedborne mosaic virus), the post-entry quarantine may be conducted in a contained site that excludes vectors in consideration of the risk (ISPM 34 *Design and operation of post-entry quarantine stations for plants*). Post-entry quarantine can be a suitable phytosanitary measure for very small lots where insufficient number of seeds are available for laboratory testing (e.g. very small lots of foundation or stock seeds imported for producing commercial seeds). In such cases, the seeds may be planted in post-entry quarantine, the plants grown from these seeds inspected for symptoms of disease or tested, and progeny seeds produced from plants that are verified as being pest-free are released for planting.

2.4 Implementing measures

The following section describes when measures can be applied, the factors to consider when implementing the measures and the types of pest risks they manage. Some measures can be applied alone to manage pest risk (e.g. PFA) while others can be used as dependent or independent measures in a systems approach for specific pests or pest groups.

2.4.1 Pre-planting

The risk of pests being introduced from the environment into the production site can be mitigated by appropriate selection, preparation and maintenance of the site. This involves assessing the status of regulated pests in the production site and selecting and implementing appropriate risk management measures while preparing the site for planting and thereafter during seed production and harvest (Table 2).

The use of healthy seeds or seeds of disease-resistant plant varieties reduce the likelihood that seedborne or seed-transmitted pests will be associated with the seeds that are used as inputs in seed production (Table 2). Seed health may be verified by testing a representative sample from the seed lot for the presence of regulated pests using screening methods that are sensitive and specific to detect pest infestation. If regulated pests are identified in the seed lot, then the seeds should not be used for seed production, unless there is an effective seed treatment available that will eliminate the pest from the seeds.

Table 2: Factors to consider during the pre-planting phase of seed production, the associated pest risks and options for phytosanitary actions to prevent or mitigate the pest risks

Factor	Pest risk	Options for phytosanitary actions
Site selection, preparation and maintenance		
The site has a history of pests or host crops.	Pests (e.g. arthropods, fungi, bacteria, viruses, and nematodes) present at the site could potentially infest the new planting for seed production.	<ul style="list-style-type: none"> Choose sites in a pest-free areas, pest-free places of production, pest-free production sites or sites in areas of low pest prevalence. 11 Monitor and identify pests at the site. Use pesticides or biological control agents for pest management. Adjust planting dates to occur in periods when pest prevalence at the site is low. Practice crop rotation or mixed cropping. Fallow the site for sufficient time to eliminate the pest.
The soil in the site is contaminated with pests.	Soilborne pests (e.g. bacteria, fungi, nematodes) could be transmitted to the new planting for seed production. Some soilborne pests (e.g. cyst nematodes) can be external contaminants in seed lots at harvest.	<ul style="list-style-type: none"> Choose sites that are pest-free areas, pest-free places of production, pest-free production sites or sites in areas of low pest prevalence. Sample, inspect and test the soil for soilborne pests. Treat the soil to control soilborne pests e.g. by soil fumigation. Use soilless growing media in greenhouse production.
The site's water source is contaminated with pests.	Waterborne pests (e.g. oomycetes such as <i>Phytophthora</i> spp., <i>Pythium</i> spp., and bacteria such as <i>Ralstonia solanacearum</i>) could be transmitted to the new planting for seed production.	<ul style="list-style-type: none"> Use clean water sources for irrigation, such as deep well water or municipal water. Sample and test the water source for waterborne pests. Filter the water to reduce pest concentration. Treat the water to control waterborne pests, e.g. by chlorination or UV treatment.

Factor	Pest risk	Options for phytosanitary actions
The crop is inadequately isolated from other crops.	Pests (e.g. arthropods, fungi, bacteria, viruses, viroids) could spread from infested hosts in and around the site to infest the new planting for seed production.	<ul style="list-style-type: none"> • Adequately isolate the new planting from other crops, considering the host status of these crops and biology of pests present in and around the site. • Isolate the new planting from all sources of pollen contamination including from wild plants that might serve as a source of disease.^[2] • Maintain a buffer zone around the site free from alternative host plants.
Weeds, volunteer plants and host plant debris are not adequately removed in and around the site.	Weeds, volunteer plants and host debris can be a source of pests transmitted to the new planting and can also contaminate seed lots at harvest.	<ul style="list-style-type: none"> • Survey the area in and around the site for weeds and volunteer plants in an ongoing manner. • Control weeds and volunteer plants by herbicide treatment or manual removal by ploughing or flaming. • Remove host plant debris from the site.
Pest and disease vectors are present in and around the site.	Vectors present in and around the site may transmit pests to the crop and increase its prevalence within the crop. For example, insect vectors could transmit virus and viroid pests to a new planting.	<ul style="list-style-type: none"> • Choose sites in pest-free areas, pest-free places of production, pest-free production sites or sites in areas of low pest prevalence. • Monitor vectors in and around the site e.g. using traps. • Use pesticides or biological control agents to manage vectors.
Hygiene measures practiced on site are inadequate to prevent or reduce pest infestation of the site.	Poor worker hygiene or the use of contaminated tools and equipment can be the source of pests that are mechanically transmitted to the new planting for seed production.	<ul style="list-style-type: none"> • Ensure that workers are trained in proper hygiene and sanitation protocols and that they sanitise their hands and footwear when entering and leaving the site. • Ensure that workers clean and disinfest their tools^[3] and field equipment before entering the site. • For crops grown in protected environments, e.g. glasshouse, sanitize the site and remove weeds and volunteer plants before and during growing seasons. Pasteurize the growing media and disinfest transplant containers, pots, planting trays and workers' tools prior to use.

Factor	Pest risk	Options for phytosanitary actions
The site is not well maintained or kept secure.	Pests can be introduced in an inadequately contained or insecure site.	<ul style="list-style-type: none"> Inspect the protected environment, e.g. glasshouse, for structural damage and repair any damage prior to planting. Adequately screen all access points in the glasshouse. Restrict access to the site by physical barriers such as gates or fencing and restrict entry to the site to authorised personnel.
Using healthy seeds or resistant plant varieties to produce seeds		
The seeds that are planted are not tested for pests.	Untested seeds that are infested with pests are planted out leading to pests transmitted to progeny seed. For example, the presence of viruses and viroids in seeds is frequently asymptomatic, but they can be transmitted to infest a new planting.	<ul style="list-style-type: none"> Use seeds from seed lots that have been tested and certified by an accredited seed testing laboratory as free from regulated pests. Phytosanitary testing of seeds may be performed as part of quality control or it may be a requirement to import the seeds into the country where seed production occurs. Use seeds that are certified as having been produced in a pest free area, pest free place of production, pest free production site or area of low pest prevalence. Keep records of the origin and use of the seeds for the purpose of traceability in the event of a pest outbreak.
The seeds that are planted are not disease resistant varieties.	The level of resistance to regulated pests is insufficient to prevent pest transmission to progeny seed.	<ul style="list-style-type: none"> Use commercial seed lines with a known history of disease resistance. Use disease resistant seed varieties as part of a suite of risk management measures such as hygiene measures.
The seeds are not effectively treated to eliminate regulated seedborne pests.	Seedborne pests (e.g. fungi and bacteria) are transmitted to the new planting for seed production.	<ul style="list-style-type: none"> Apply appropriate seed treatments to prevent the infestation of seedlings with seedborne and soilborne pests. The treatment should be effective against the pest and applied at an efficacious dose. Apply seed treatments as part of quality control or to meet the phytosanitary requirements to import the seeds into the country where seed production occurs. Retest the seeds to verify that the treatment is effective, and the pathogen is eliminated from the seed e.g. pathogen viability assay.

^[1] In the USA, seed-growing has been shifted to dry Pacific regions for crops such as cabbages, turnips, beans, and peas to obtain disease-free seed and indirectly control diseases as black leg and black rot of cabbage and turnip, etc. (Gupta and Kashyap, 2020)

^[2] For the production of certified capsicum seed, plants should be grown at a minimum distance of 250 m from sources of pollen contamination and seedborne diseases.

^[3] An example of disinfecting tools is by washing them in soapy water and then wiping them with or dipping them in disinfectant e.g. 70% ethanol.

2.4.2 Pre-harvest

Pre-harvest measures are used from the time the seeds are planted in the site to the time the crop has grown and set seed and is ready to be harvested. The measures reduce the likelihood that the seed produced will be infested by pests that are transmitted to them from the growing plants.

The measures include the use of hygiene practices in the production site, crop treatment, field inspection and parent plant testing (Table 3). Where the measure is undertaken to meet a phytosanitary requirement of an importing country (e.g. field inspection or parent plant testing), the NPPO of the seed producing country is responsible for oversight and verification of the measure prior to providing phytosanitary certification for the exported seed.

Table 3: Factors to consider during the pre-harvest phase of seed production, the associated pest risks, and options for phytosanitary actions to prevent or mitigate the pest risks

Factor	Pest risk	Options for phytosanitary actions
Hygiene practices in the production site		
Hygiene measures practiced on site are not adequate to prevent or control pests infesting the new plants.	Poor worker hygiene, an inadequately sanitized field or the use of contaminated tools and equipment can be sources of pest infestation for new plants planted for seed production. For example, tobamoviruses such as tomato brown rugose fruit virus can easily spread in tomato and capsicum crops through virus-contaminated tools.	<ul style="list-style-type: none"> • Ensure that workers are trained in proper sanitation and hygiene protocols and that they sanitize their hands and footwear when entering and leaving the site. • Clean and sanitize tools and field equipment including tractors and planters before entering the site. • Use clean water sources for irrigation such as deep well water or municipal water. Disinfest water sources before use by chlorination or UV treatment. • Remove infested plants as soon as symptoms appear, bag them and dispose of them away from the site. Remove infested fruit and plant debris to reduce the pathogen inoculum that could come into contact with healthy plants. • Dispose of cull piles away from production fields and waterways. Cover cull piles with sheet plastic to speed up microbial decomposition and prevent pathogen spores from escaping. • Control weeds and volunteer plants within the site using herbicides or remove them manually.

Factor	Pest risk	Options for phytosanitary actions
Crop treatment		
The crop is not adequately treated to effectively control pests infesting the new plants	The new plants are infested by pests, which are transmitted to the progeny seeds.	<ul style="list-style-type: none"> • Apply crop treatments to the new planting as a preventative measure (i.e., to prevent infestation of the plant) or curative measure (i.e., to reduce or eliminate the level of infestation in the plant). • Apply appropriate chemical or biological pesticides on the crop depending on the crop, pest biology and mode of action. Most crop treatments are used to manage bacterial and fungal pests.^[1]
Field inspection		
Pests infest the new plants during growing season. Field inspection is inadequate to identify pest infestation during growing season.	The new plants can be infested by seedborne pests and non-seedborne pests that may be present in the environment	<ul style="list-style-type: none"> • Conduct field inspection of all plants in the site at an appropriate time during the growing season, for regulated pests that cause visual symptoms. Field inspection can also be conducted to monitor for regulated pests and vectors in the field. • Ensure that field inspections are performed by personnel trained in identifying and diagnosing symptomatic plants and disposing infested plants using appropriate methods. • Ensure that the field inspection pattern selected adequately and proportionately represents all parts of the field. All parts and rows should be covered and crossed by the inspector on foot. The walking in the field should be done in a systematic pattern so that the maximum area possible can be covered e.g. following the walking patterns for field inspection described in Feistritz (1975). • Where visible symptoms are detected during a field inspection, take an appropriate number of samples from suspected plant parts for laboratory identification, using appropriate diagnostic methods. • Label the plant samples to identify the crop, plant part, date, time and location of collection and name of the collector. Store the samples appropriately. • If field inspection is a phytosanitary requirement of the importing country, the NPPO of the seed producing country should ensure/verify that field inspection has been conducted in accordance with the requirements of the importing country e.g. through audits.

Factor	Pest risk	Options for phytosanitary actions
Parent plant testing		
Parent plant testing is inadequate to identify pest infestation.	Asymptomatically infested parent plants produce infested progeny seeds.	<ul style="list-style-type: none"> • Sample and test actively growing parent plants for the presence of regulated pests that could cause asymptomatic infestation in these plants and be transmitted to the progeny seeds. • Test an appropriate number of parent plants from the site to identify at a specific confidence level that a specific infestation level of a pest will be detected in the parent plants. • Label suspect infested plant samples to identify the crop, plant part, date, time and location of collection and name of the collector and store the samples appropriately. • Ensure that appropriate measures are in place to prevent infestation of plants post testing e.g. through vectors. • Parent plant testing could be used alone or in combination with field inspection and may be required to meet an importing country's phytosanitary requirements.

¹¹ Systemic fungicides, such as the sterol biosynthesis inhibiting (SBI) and demethylation inhibiting (DMI) fungicides, diffuse into the plant tissues to some extent and eliminate recently established infestations (Maloy, 2005).

2.4.3 At harvest

The risk of pests being introduced and contaminating seeds at the time of harvest can be prevented or mitigated by implementing hygiene practices during harvest, selecting an appropriate time of harvest and keeping the harvested seeds protected from potential sources of external contamination. Table 4 describes measures that may be used to minimize seed contamination at the time of harvest in the production site.

Table 4: Factors to consider during harvest phase of seed production, the associated pest risks and options for phytosanitary actions to prevent or mitigate the pest risks

Factor	Pest risk	Options for phytosanitary actions
Timing of harvest		
Seeds are contaminated at the time of harvest due to inappropriate timing of the harvest.	Pests infest the harvested seeds when harvest occurs at an unfavourable time, e.g. when pest populations in the site are high, during unfavourable weather conducive to disease development or when plants are at a susceptible stage for seed infestation.	<ul style="list-style-type: none"> • Monitor pest populations and disease epidemiology in the site. • Avoid harvesting the seeds during wet weather conditions that are conducive to disease development and contamination of the seeds. • Consider pest incidence in the site, crop and seed maturity and weather conditions when timing the harvest.

Factor	Pest risk	Options for phytosanitary actions
Hygiene measures		
Hygiene measures practiced on site are inadequate to prevent pests contaminating seeds at harvest.	Seeds are contaminated during harvest due to poor hygiene and sanitation practices.	<ul style="list-style-type: none"> • Ensure that workers are trained in hygiene and sanitation protocols to apply during harvest to prevent contamination of seeds. • Clean and sanitize tools and equipment including seed extraction and drying equipment before use during harvest. • Use disinfectants during seed extraction. • Dry seeds immediately after extraction to reduce moisture content of the seeds. • Store the harvested seed in sealed, new or sanitized containers or bags. • Clean the site and remove, burn, chop or deep plough plant debris after harvest.

2.4.4 Post-harvest

2.4.4.1 Seed cleaning

Harvested seeds should be subjected to a cleaning process undertaken in a manner that prevents mixing of batches as well as cross-contamination of healthy batches with potentially infested seeds and plant parts.

The harvested seeds should be separated from all debris (non-seed material) that may harbour pests, seeds showing signs of damage or pest infestation and seeds of other species (including weed seeds). Non-seed material (e.g. sticks, leaves, dirt, stones) and weed seed contaminants can be separated from the harvested seeds based on differences in size and shape, weight or surface texture. For example, contaminants can be separated from the harvested seed based on differences in size and shape by scalping (using a screen which allows the harvested seed to fall through a round or rectangular sieve while larger particles are excluded) or sieving (using a screen sieve to sieve out particles smaller than the seed while retaining the seed). Separations based on differences in weight, specific gravity or surface area can be made using a box fan, air column, aspirator or pneumatic air separator. These work by passing a stream of air past the seed allowing the light often non-viable seed and light chaff to be blown out of the lot. Harvested seed may also be separated from contaminants based on surface texture differences.

Seed cleaning should be done immediately after harvest to prevent the likelihood of pests associated with debris from infesting the harvested seeds. Equipment used in the seed cleaning process (e.g. graduated sieves, seed blower) should be cleaned or disinfested between runs to prevent cross-contamination of each batch of seed. The cleaned seeds may be inspected to verify that they are free from visually detectable extraneous contaminants and pests.

2.4.4.2 Physical seed treatments (e.g. dry heat, steam, hot water)

Physical treatments include heat treatment and irradiation. The most common heat treatments are hot water and hot air. Heat treatment may be used to inactivate or kill some seedborne pathogens but can impact seed viability. Hot water treatment is a long-known technique that consists of immersing plant material in agitated water at a predetermined temperature and time. Both hot water and dry heat treatment may be effective disinfestation methods for controlling some seedborne diseases in vegetables, although the treatment should be carefully performed to maintain treatment efficacy and minimise impact on the germinability of seeds. In general, hot water treatment of older seeds treated under the same conditions may show reduced germination compared to fresh seed. Hot water treatment should be performed after checking the age and condition of the seeds.

Table 5: Examples of some common hot water treatment for selected crops and pests

Vegetable/Pests	Temperature	Time (minutes)	Helps protect against
Brassica/canker	50 °C	30	<i>Leptosphaeria maculans</i>
Brassica/black rot	50°C	30	<i>Xanthomonas campestris</i> pv. <i>campestris</i>
Tomato/canker	53 °C	60	<i>Clavibacter michiganensis</i> subsp. <i>Michiganensis</i>
Pea	55 °C	15	<i>Pseudomonas syringae</i> pv. <i>pisii</i>

Source: Modified from Van der Wolf, J.M. 2006. *Seed health management*. [Cited 18 October 2022]. <https://edepot.wur.nl/3616>

2.4.4.3 Chemical seed treatments (pesticides, disinfestants)

Pesticides are mainly applied to manage fungi, bacteria, insects, and nematodes associated with seeds. The treatment method is performed according to the seed type and target pests for each pesticide. Chemical treatments may be applied during seed processing (e.g. seed extraction or seed priming) or a dedicated disinfestation process. When treating vegetable seeds, it is critical to follow the directions exactly. If the directions are not followed, germination can be reduced by the treatment or the pests may not be eliminated. It is a good idea to trial the treatment on a small portion of seeds to determine the effect on germination before treating a large amount of seeds. It is also important to apply the chemical properly. Too much of the chemical can result in phytotoxicity, and inadequate coverage can result in incomplete treatment. The chemical used for seed treatment may need to be approved by, or provide an equivalent level of protection as required by the importing country.

2.4.4.4 Biological seed treatments (e.g. plant extracts, natural compounds, biocontrol agents)

Biological treatment is a method of controlling or suppressing seedborne pests using plant extracts containing natural antimicrobial compounds or numerous microorganisms as biocontrol agents. In some circumstances, this method may be used as an alternative to pesticide treatment or in combination with physical treatment. Acceptable modes of action for biological control agents are antagonistic (antibiotic), competitive (niche exclusion), and induced system resistance.

Table 6: Examples of common vegetable seed treatment products registered for use in the United States as at April 2020

Active ingredient	Helps protect against
Bactericides	
Streptomycin sulphate	Halo blight of bean
Fungicides	
Azoxystrobin	Seedborne and soilborne fungi that cause decay, damping-off and seedling blight including species of <i>Rhizoctonia</i> and <i>Penicillium</i> , and some protection from seedborne head smut on sweet corn
Captan	Seedborne and soilborne fungi that cause decay, damping-off and seedling blight
Carboxin	Seed decay, fungi that cause damping-off including <i>Rhizoctonia solani</i> and seedborne smut of corn
Carboxin+thiran	Onion smut
Fludioxonil	Seedborne and soilborne fungi that cause decay, damping-off and seedling blight
Iprodione	<i>Alternaria</i> species
Mefenoxam	Damping-off and seed rots caused by <i>Pythium</i> , systemic downy mildew in garden pea and sweet corn
Mefenoxam+difenoconazole	Suppression of post emergent die-back complex and damping-off
Metalaxyl	<i>Pythium</i> damping-off, early season <i>Phytophthora</i> , systemic downy mildew in bean
Thiabendazole	Seedling disease caused by <i>Fusarium</i> spp., <i>Verticillium</i> wilt of spinach, <i>Phomopsis</i> seed decay, seedling wilt, damping-off of bean, <i>Ascochyta</i> blight of pea
Thiram	Seed decay, damping-off, seedling blight caused by many seedborne and soilborne organisms
Insecticides	
Clothianidin	Chinch bug, corn flea beetle, cutworm, seed corn maggot, southern corn rootworm, wireworm
Imidacloprid	Seed corn maggot, fire-ant, wireworm
Spinosad	Onion maggot and seed corn maggot
Thiamethoxam	Certain seed corn maggots, wireworm
Abamectin	Root-knot nematode

Source: Seminis. 2020. *Vegetable Seed Treatment*. Agronomic Spotlight.

Seed treatment may be used as a single measure or in combination with other measures to ensure pest risks are mitigated to an acceptable level. A combination of seed treatments can also be applied. The seed producers or manufacturers should keep a seed treatment record, and this should be available for the NPPOs of the exporting or importing country if requested.

2.4.5 Pre-export

2.4.5.1 Sampling and methodology

It is important to apply good sampling methodologies to ensure the samples represent the characteristics of the seed lot or consignment. A representative sample enhances the accuracy and acceptance of the inspection and test results.

When selecting a sampling methodology, parameters to consider may include:

- objective of the sampling (e.g. for inspection, purity testing or seed health testing);
- level of homogeneity of the seed lot or consignment;
- required accuracy of the inspection or test results, which is dependent on level of detection, confidence level and efficacy of detection; and
- what the inspection or test results are to be accepted (e.g. for a seed lot, seed consignment or just the submitted sample).

Some importing countries may prescribe specific sampling requirements for which the inspection or testing is conducted.

Guidance in selecting appropriate sampling methodologies for inspection or testing of consignments to verify compliance with phytosanitary requirements is given in ISPM 31 (*Methodologies for sampling of consignments*). Seed testing organizations such as the International Seed Testing Association (ISTA) have developed seed sampling rules and procedures for specific purposes, which may provide NPPOs with additional guidance on potential sampling methodologies for those specific purposes.

2.4.5.2 Sample size

Sample size is usually determined to provide a specific confidence level that a specific infestation rate or higher (detection level) of a pest within a seed lot or consignment will be detected. At a specific pest infestation rate, the confidence level of pest detection usually increases as sample size increases. However, for a seed lot or consignment that is homogeneous, increasing sample size beyond a certain size will not be necessary to detect a given threshold of pest contamination at a given level of confidence.

The required sample size may vary depending on what the sample will be used for, e.g. phytosanitary inspection, purity testing or seed health testing.

For seed health testing, when taking into consideration the efficacy of detection methods, the specific confidence level and the specific detection level may be determined by acceptable level of pest risk, which may be based on the regulated pest species and the appropriate level of protection of the importing country. Some importing countries may prescribe required sample size (or subsample size) for seed health testing of a specific regulated pest using a specific testing method.

Seed may be moved or traded in small quantities, commonly referred to as a ‘small seed lot’. The usual required sample size may account for a large proportion of, or even larger than, the small seed lot. Destructive analysis, such as that required by seed health testing, on a large proportion of a seed lot may not be practical, particularly for high-value seeds or breeder seeds. Therefore, the required sample size for a small seed lot may be different from a large seed lot. For example, the sample size for a small seed lot may be calculated for hypergeometric-based sampling (as outlined in Appendix 2, ISPM 31) or set as a specific percentage of the small seed lot. The description of and the required sample size for a small seed lot may, however, vary among different seed species and importing countries.

ISPM 31 has more guidance on determining sample sizes.

2.4.5.3 Inspection and purity testing

Pre-export inspection is usually required by importing countries to ensure that seed consignments are compliant with the phytosanitary requirements of importing countries at the

time of inspection. The exporting country's NPPO is responsible for pre-export inspection. Inspections may be carried out by the exporting country's NPPO or by personnel or agents authorized by the NPPO. If a consignment comprises more than one seed species or lot, inspection may need to be performed separately for each of the seed species or lots within the consignment. Inspection may involve visual inspection (of the entire consignment or representative sample of seeds from the consignment) to detect the presence of regulated pests and regulated articles, examination of documents associated with the consignment, and verification of identity of the consignments.

Visual inspection is often used to detect contaminants (such as contaminant seeds, soil, fungal bodies, arthropod pests, plant debris and animal matters/parts) within the consignment. Visual inspection can also be used to detect the presence of some regulated pests such as external pests and pests that cause visually detectable signs or symptoms.

Visual inspection methods should be designed to detect contaminants and targeted pests. Inspectors should have technical qualifications or competency and capability in pest detection, which can be demonstrated through training records and the ability on the use of materials and equipment.

Procedures for the inspection of consignments of plants and plant products are described in ISPM 23 (*Guidelines for inspection*).

Some contaminants are regulated pests such as weed seeds, and some contaminants are regulated articles such as soil. Visual inspection may not be sufficient to detect the presence of some contaminants, particularly for large seed lots containing small seed species. For these, some importing countries may require purity testing, as part of their phytosanitary requirements, to determine the presence/absence and, if present the percentage and identity of, contaminants within a seed lot. Procedures for seed purity testing and accreditation system for approval of service providers/laboratories established by certain organizations, for example ISTA are generally recognized by NPPOs.

2.4.5.4 Seed testing

Similarly, visual inspection may not be sufficient to detect pests that may not produce visible symptoms or signs of infestation such as some bacteria, fungi, viruses and viroids. Seed health testing using diagnostic methods such as molecular or serological methods may be required to detect and identify these pests. Diagnostic methods are selected and validated to ensure sensitivity, specificity and reproducibility for detecting regulated pests in the sample. Diagnostic methods validated on other plant tissues may not necessarily be effective for seeds and therefore validation of the methods on specific seed species should be conducted. The use of robust diagnostic methods and procedures is critical to ensure the accuracy of the seed health test, while preventing false positive or false negative pest identification results. Diagnostic protocols provide the minimum requirements, including diagnostic methods and procedures, to reliably identify regulated pests.

Guidance on the diagnostic protocols that describe methods and procedures for the diagnosis of regulated pests is provided in ISPM 27 (*Diagnostic protocols for regulated pests*). Adopted diagnostic protocols for some regulated pests are provided as annexes to ISPM 27. Information on seed testing methods for a range of seedborne and seed-transmitted pests is also available in ISTA Seed Health Methods and the International Seed Health Initiative for Vegetable Crops (ISHI-Veg) under the International Seed Federation (ISF). It is important to note that diagnostic protocols for regulated pests are subject to review and therefore may change from time to time to take into account new developments in pest diagnosis.

Some countries may have specific requirements for inspection and testing of pelleted seeds or treated seeds. Guidance on inspection of pelleted seeds and testing of treated seeds is provided in ISPM 38.

3. Phytosanitary certification

Before issuing a phytosanitary certificate for export, the NPPO of the exporting country will need to examine and confirm that the seed consignment complies with the import requirements of the importing country. A phytosanitary certificate should include, at the minimum, information on the scientific name and country of origin of individual seed lots within the consignment. Including a unique identifier of individual seed lots, such as seed lot number, is also important for traceability. A phytosanitary certificate may also need to include additional declarations to certify that the seed consignment is free from regulated pests, for example, is sourced from a PFA or a PFPP or has been tested or treated in accordance with the requirements of the importing country. Some importing countries may also require laboratory reports to accompany the consignment.

A phytosanitary certificate for re-export can be issued by the NPPO of the re-exporting country where an original phytosanitary certificate for export or a certified copy is available. The phytosanitary certificate for re-export should include traceability to the phytosanitary certificate issued by the country of origin and take into account any changes in the pest risk associated with the consignment that may have occurred in the country of re-export (e.g. through processes which may expose the consignment to infestation or contamination by pests, such as repackaging). The final destination country for seed may not be known when the original phytosanitary certificate is issued meaning that the certificate may not include all required assurances. When this occurs the NPPO of the country of origin may need to provide these assurances.

Requirements and guidelines for the preparation and issuance of phytosanitary certificates for export and phytosanitary certificates for re-export are provided in ISPM 12 (*Phytosanitary certificate*).

4. Responsibilities of the NPPO of the seed-producing country

If required to meet importing country requirements, the NPPO of the origin country of the seeds is responsible for:

- 1) Having oversight of organizations involved in seed production by, for example, registering and auditing seed producers using a systems approach to manage pest risk, authorizing entities to conduct specific phytosanitary actions (as per ISPM 45 *Requirements for national plant protection organizations if authorizing entities to perform phytosanitary actions*).
- 2) Having systems in place for preventing the introduction of new pests and diseases into the country. Such systems could integrate elements including PRA, post-entry quarantine, treatment of imported seeds etc. A country may use post-entry quarantine to grow out seeds and verify that the plants produced from the seeds are free from pests and diseases. If the destination country of the seeds is known, the NPPO should confirm the seeds are free from quarantine pests and pests of concern to these countries before exporting the seeds. If the destination country of the seeds is not known at the time of import, countries may generally ensure that pests of concern to trading partners where the seeds could potentially be exported are managed.

- 3) Maintaining procedures and competency for authorized personnel to ensure they have work capabilities in performing phytosanitary actions such as seed sampling, seed inspection and field inspection.
- 4) Approving the pest management practices used in seed production or the control system for seed processing by producers or manufacturers to ensure that the risks of quarantine pests have been mitigated.
- 5) Monitoring or auditing production measures in the field, and the control system of producers or manufacturers during their operational seasons to ensure their continued compliance with requirements, pest management practices and the requirements of the importing countries or destination countries. Frequency and timing of the monitoring or auditing should be determined according to the pest risks, phytosanitary import requirements and history of conformity and compliance.
- 6) Field inspection, testing of suspected plants and verifying the documentation and pest management practices.
- 7) Inspecting, sampling and testing consignments of seed for export.
- 8) Confirming the phytosanitary requirements of the destination country and only issuing phytosanitary certificates to confirm that all seed consignments being exported comply with them.
- 9) Maintaining phytosanitary security of the certified seed to the point of export.
- 10) Managing investigations into potential non-compliances reported on the pathway.

APPENDIX 1: CAPSICUM SEEDS

This appendix only applies to *Capsicum* spp. (Solanales: Solanaceae) seeds for planting. It does not apply to vegetative plant parts of species under this genus. The pests and measures are not exhaustive and equivalent options for measures can be considered. The specific quarantine pests requiring risk mitigation measures and the measures required to achieve an appropriate level of protection will likely differ for each importing country.

1. Background

Capsicum is an economically important genus of the Solanaceae family, which contains at least 32 species native to tropical America and is grown commercially from seeds. Five species are widely cultivated in different regions of the world: *C. annuum* L., *C. chinense*, *C. frutescens* L., *C. baccatum* L. and *C. pubescens*. *Capsicum* seeds can carry pests such as fungi, bacteria, viruses and viroids and the diseases caused by these pests can cause significant reduction in fruit yield and seed production.

2. List of seedborne and seed-transmitted pests associated with capsicum seeds

This is an illustrative list of pests known to be associated with capsicum seeds and regulated by at least one country. It is not exhaustive. Inclusion of a pest in this list does not constitute technical justification for its regulation. Also, additional pests may be considered as requiring phytosanitary measures. When an importing country is determining whether to regulate a pest on capsicum seeds for sowing, decision-makers should use either a PRA, or where applicable, another comparable examination and evaluation of available scientific evidence.

Table 1: Illustrative pest groups that may be associated with capsicum seeds

Pest group	Family	Example species
Fungi	Glomerellaceae	<i>Colletotrichum actuatium</i> <i>Colletotrichum capsici</i> <i>Colletotrichum gleosporioides</i> <i>Colletotrichum coccodes</i>
Bacteria	Xanthomonadaceae	<i>Xanthomonas euvesicatoria</i> <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> <i>Xanthomonas gardneri</i> <i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>
Viruses	Virgaviridae	<i>Pepper mild mottle virus</i> <i>Tobacco mosaic virus</i> <i>Tomato mosaic virus</i> <i>Tomato brown rugose fruit virus</i> <i>Tomato mottle mosaic virus</i>
Viroid	Pospiviroidae	<i>Potato spindle tuber viroid</i> <i>Pepper chat fruit viroid</i> <i>Columnnea latent viroid</i>

3. Managing seedborne pathogens associated with capsicum seeds

3.1 Fungi

One strategy to prevent outbreaks of disease in the seedbed or field is to use healthy seeds. Seeds can be tested to verify that they are free from fungi, or physical or chemical treatments can be applied to the seeds before planting. A sample of seeds can be verified to be free from *Colletotrichum* species by plating them on potato dextrose agar (PDA) (Chigoziri and Ekefan, 2013), or by using the standard blotter method (Welideniya *et al.*, 2019), or by using polymerase chain reaction (PCR) with *Colletotrichum* species-specific primers (Dhiman *et al.*, 2022). Some seeds can be treated with hot water as a preventive measure to reduce the incidence of seedborne fungi (Lewis Ivey, 2013).

Plants can be inspected and/or tested in the field at different growing stages for signs of disease. Typical symptoms of anthracnose disease on capsicum fruit are circular or angular sunken lesions that produce pink to orange conidial masses. (Lewis Ivey *et al.*, 2004). Diseased plants can be removed because they can be a source of inoculum. Plants can be well spaced to prevent becoming infested by fungal spores that can be transmitted over short distances by water splash. Plants should be watered and where possible without extreme humidity that promotes fungal growth.

Healthy plants are generally less susceptible to disease. To promote healthy plant growth, fertilize plants with a balanced mix of potassium phosphate and magnesium and avoid over-fertilizing with nitrogen. The chemical fungicides that are generally recommended for controlling anthracnose disease are based on copper compounds, dithiocarbamates, benzimidazole and triazole compounds (Waller, 1992). Manganese ethylene bis (dithiocarbamate) (e.g. maneb) (Smith, 2000) and carbendazim can be used to control mild infestations of anthracnose disease, but are ineffective during severe disease outbreaks. It is a good practice to spray plants with fungicides such as maneb at the beginning of flowering to control early infestations on fruit (Sheu, Wang and Jackson, 2022). Seeds can be harvested from healthy fruit. Harvested seeds can be surface-sterilized to remove or reduce surface fungal contamination. For example, surface sterilisation for 3 minutes with 1.0% sodium hypochlorite is recommended by Chigoziri and Ekefan (2013).

Table 2: Examples of strategies for managing some fungal diseases of capsicum

Management strategy	<i>Colletotrichum actuatum</i>	<i>Colletotrichum capsici</i>	<i>Colletotrichum coccodes</i>	<i>Colletotrichum gleosporioides</i>
Use resistant cultivars	✓	✓	unknown	unknown
Plant disease-free or treated seeds	✓	✓	✓	✓
Rotate crops	✓	✓	✓	✓
Avoid handling seedlings	✓	✓	✓	✓
Control vectors	X	X	X	X
Use greenhouse sanitation	✓	✓	✓	✓

Management strategy	<i>Colletotrichum actuatum</i>	<i>Colletotrichum capsici</i>	<i>Colletotrichum coccodes</i>	<i>Colletotrichum gleosporioides</i>
Inspect crop for signs of disease	✓	✓	✓	✓
Remove infested seedlings and plants	✓	✓	✓	✓
Eliminate volunteer and weed hosts	X	X	X	X
Avoid handling and injuring plants in the field	✓	✓	✓	✓
Spray crop for chemical control	✓	✓	✓	✓
Harvest seeds from healthy fruit	✓	✓	✓	✓
Clean and disinfest harvested seeds	✓	✓	✓	✓
Destroy crop promptly after harvest	✓	✓	✓	✓

✓ an option for managing pest risk either alone or in combination with one or more measures

X maybe inappropriate or ineffective management option

Unknown if the option is effective at managing the pest risk

3.2 Bacteria

A strategy to prevent outbreaks of bacterial disease in the seedbed or field involves using healthy seeds and rotating sites used as seedbeds. It is important to maintain strict hygiene precautions such as sterilizing soil (e.g. steaming seedbeds) and disinfesting tools (e.g. washing in soaping water and wiping or dipping in 70% ethanol). If infested plants are found in the seedbed, they can be destroyed.

Most seedborne bacteria, such as *Xanthomonas* spp., are either endemic or introduced into production areas through infested seed or transplant materials (Jones *et al.*, 1986; Sijam, Chang, and Gitaitis, 1991) (e.g. transplanted seedlings). The primary management strategy can include the use of pathogen-free, certified seeds or disease-free transplant materials (Ritchie, 2000). There is a validated seed health test that can be used to verify that seeds are free from *Xanthomonas euvesicatoria*, *Xanthomonas vesicatoria* and *Xanthomonas gardneri* (ISF, 2017). Seeds can be treated in some circumstance with hot water as a preventive measure or to reduce the incidence of seedborne bacteria (Lewis Ivey, 2013).

Early recognition of disease symptoms is essential for preventing diseases from spreading in a crop. Crop inspections can be undertaken at different growing stages. Inspectors can look for disease symptoms, which include leaf spots, leaf lesions with chlorosis, wilting and bird's eye spots on fruit. Potential sources of inoculum sources, such as volunteer plants and infested host plants, can be removed promptly. Infested crops can be rotated with non-host crops for at least two years to reduce the incidence of bacterial canker (Goode and Sasser, 1980; Ritchie, 2000).

Chemical control has been a primary tool of pest management strategies for bacterial spot of tomato and capsicum. Applying copper-based chemicals to plants in the field may slow secondary bacterial canker development (Hausbeck *et al.*, 2000). Treating the harvested seeds

with a solution of chlorine bleach can in some circumstances be used to remove or reduce bacterial pathogens from the seed surface (Lewis Ivey, 2013).

Table 3: Examples of strategies for managing some bacterial diseases of capsicum

Management strategy	<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>	<i>Xanthomonas euvesicatoria</i>	<i>X. campestris</i> pv. <i>vesicatoria</i>	<i>X. gardneri</i>
Use resistant cultivars	unknown	unknown	✓	unknown
Plant disease-free or treated seeds	✓	✓	✓	✓
Rotate crops	✓	✓	✓	✓
Avoid handling seedlings	✓	✓	✓	✓
Control vectors	X	X	X	X
Use greenhouse sanitation	✓	✓	✓	✓
Inspect crop for signs of disease	✓	✓	✓	✓
Remove infested seedlings and plants	✓	✓	✓	✓
Eliminate volunteer and weed hosts	✓	✓	✓	✓
Avoid handling and injuring plants in the field	✓	✓	✓	✓
Spray crop with copper-based chemicals	✓	✓	✓	✓
Harvest seeds from healthy fruit	✓	✓	✓	✓
Clean and disinfest harvested seeds	✓	✓	✓	✓
Destroy crop promptly after harvest	✓	✓	✓	✓

3.3 Viruses

The main strategy to prevent outbreaks of viral disease in the seedbed or field involves using healthy seed, inspecting the crop for signs of disease and removing diseased plants, which can be sources of inoculum. Seeds for planting can be produced in areas with a pest status of absent or PFAs or tested to verify that they are free from viruses. Seeds can in some cases be tested using enzyme-linked immunosorbent assay (ELISA) or PCR assays to verify they are free from pathogens, such as pepper mild mottle virus, tobacco mosaic virus, tomato mosaic virus (ISF 2019a), tomato brown rugose fruit virus (ISF 2019b) and tomato mottle mosaic virus (Levitzky *et al.*, 2019).

Healthy plants are generally less susceptible to disease. To promote plant health, plants can be fertilized with well-balanced nutrients potassium phosphate and magnesium, and over-fertilizing with nitrogen should be avoided. Plants can be inspected at different growing stages for expression of symptoms of viral disease, which can include foliar mottle, chlorosis or mosaic, leaf distortion, defoliation, discoloured fruit, reduced fruit size, and stunted growth (Arogundade *et al.*, 2020).

The appropriate control strategy should be based on the transmission characteristics of the virus. For example, hygiene precautions including minimising handling of seedlings, sanitizing the greenhouse and workers' tools and equipment used for propagation are important for minimising opportunity for mechanically transmitted tobamoviruses such as tobacco mosaic virus, tomato mosaic virus and tomato brown rugose fruit virus (Arogundade *et al.*, 2020).

Soil can be kept free from plant debris as tobamoviruses are very stable and can stay infectious in soil and plant debris for several year. Contact between the plants and machinery used in cultural operations can be avoided, and tools, propagating material and equipment can be disinfested with sodium hypochlorite (1 percent solution of a 12 percent concentrate of pool chlorine) to minimise opportunity for virus spread in the crop or beyond. Reservoir hosts for solanaceous-infesting viruses including *Chenopodium murale*, *Chenopodium quinoa*, *Petunia* hybrids and wild tomato species (ISF, 2019) can be removed from in and around the production site.

Table 4: Examples of strategies for managing some viral diseases of capsicum

Management strategy	Pepper mild mottle virus	Tobacco mosaic virus	Tomato mosaic virus	Tomato brown rugose fruit virus	Tomato mottle mosaic virus
Use resistant cultivars	✓	✓	✓	✓	unknown
Plant disease-free or treated seeds	✓	✓	✓	✓	✓
Rotate crops	✓	✓	✓	X	X
Avoid handling seedlings	✓	✓	✓	✓	✓
Control vectors	X	X	X	X	X
Use greenhouse sanitation	✓	✓	✓	✓	✓
Inspect crop for signs of disease	✓	✓	✓	✓	✓
Remove infested seedlings and plants	✓	✓	✓	✓	✓
Eliminate volunteer and weed hosts	✓	✓	✓	✓	✓
Avoid handling and injuring plants in the field	✓	✓	✓	✓	✓
Spray crop for chemical control	X	X	X	X	X

Management strategy	Pepper mild mottle virus	Tobacco mosaic virus	Tomato mosaic virus	Tomato brown rugose fruit virus	Tomato mottle mosaic virus
Harvest seeds from healthy fruit	✓	✓	✓	✓	✓
Clean and disinfest harvested seeds.	✓	✓	✓	✓	✓
Destroy crop promptly after harvest.	✓	✓	✓	✓	✓

3.4 Viroids

The main strategy to prevent outbreaks of disease in the seedbed or field is using healthy seeds and good crop sanitation practices. Viroidal infestations of a crop can be prevented by planting viroid-free seeds that are produced in pest-free areas, or by testing seed inputs to verify they are free from viroids. For example, validated PCR tests are available to detect potato spindle tuber viroid and pepper chat fruit viroid in capsicum seeds (EPPO, 2021; Verhoeven *et al.*, 2004).

Viroids can be mechanically transmitted by contaminated machinery, tools and people that have been in contact with infested plants and by insect vectors such as aphids and bumblebees. Disinfest machinery, tools, workers' hands and footwear. Grow seedlings in an insect-proof greenhouse and control vectors using insecticides to prevent the virus spreading in a crop. Some weedy solanaceous plants (e.g. *Solanum nigrum*) can act as hosts of viroids. Removing these plants from in and around the production site by roguing will reduce the opportunities for viroids to spread to or from the crop by vectors.

Viroids may infest a crop symptomatically or asymptotically, depending on the host cultivar. Symptomatic infestations can be detected early by inspecting seedlings and plants at different growing stages and roguing diseased plants. Infested plants and plant debris can be removed from the greenhouse or field and destroyed by composting. The greenhouse and the tools and equipment used can be thoroughly disinfested before the next planting.

Table 5: Examples of strategies for managing some viroid diseases of capsicum

Management strategy	Potato spindle tuber viroid	Pepper chat fruit viroid	Columnnea latent viroid
Use resistant cultivars	✓	unknown	unknown
Plant disease-free seeds	✓	✓	✓
Rotate crops	X	X	X
Avoid handling seedlings	✓	✓	✓
Control vectors	✓	✓	✓
Use greenhouse sanitation	✓	✓	✓
Inspect crop for signs of disease	✓	✓	✓

Management strategy	Potato spindle tuber viroid	Pepper chat fruit viroid	Columnnea latent viroid
Remove infested seedlings and plants	✓	✓	✓
Eliminate volunteer and weed hosts	✓	✓	✓
Avoid handling and injuring plants in the field	✓	✓	✓
Spray crop for chemical control	X	X	X
Harvest seeds from healthy fruit	✓	✓	✓
Clean and disinfest harvested seeds	✓	✓	✓
Destroy crop promptly after harvest	✓	✓	✓

APPENDIX 2: BIBLIOGRAPHY

References

- APSA (Asia and Pacific Seed Association).** 2022. [Cited 14 October 2022]. <https://web.apsaseed.org/asia-pacific-seed-trade-reeling-from-covid-19-lockdown-survey>
- APSA & ISF (International Seed Federation).** 2017. *Vegetable Seed Production Good Practice Guide*. APSA Crop Group Vegetables and Ornamentals and ISF Working Group Vegetable Seed Production. 9 pp.
- Arogundade O., Ajose, T., Osijo, I., Onyeanusi, H., Matthew, J., & Aliyu, T. H.** 2020. Management of viruses and viral diseases of pepper (*Capsicum* spp.) in Africa. In A. Dekebo (ed.) *Capsicum*. London, UK, IntechOpen. <https://doi.org/10.5772/intechopen.92266>
- Chigoziri, E. & Ekefan, E.J.** 2013. Seed borne fungi of chilli pepper (*Capsicum frutescens*) from pepper producing areas of Benue State, Nigeria. *Agriculture and Biology Journal of North America*. 4(4): 370–374.
- Dhiman, S., Kumari, N., Badiyal, A., Sharma, V. & Sharma, P.N.** 2022. Development and validation of a direct PCR based assay for the detection of *Colletotrichum* species on chilli seeds. *Seed Science and Technology*. 50(1): 149–161.
- EPPO (European and Mediterranean Plant Protection Organization).** 2021. EPPO Standard PM 7/138 Pospiviroids (genus *Pospiviroid*). EPPO Bulletin. 51(1): 144–177.
- FAO.** 2019. *Guide for establishing and maintaining pest free areas*. Rome. Published by FAO on behalf of the Secretariat of the International Plant Protection Convention (IPPC). [Cited 12 August 2022]. <https://www.fao.org/3/ca5844en/CA5844EN.pdf>
- Feistritzer, W.P.** 1975. *Cereal Seed Technology: A Manual of Cereal Seed Production, Quality Control, and Distribution*. FAO Agricultural Development, Paper No. 98. FAO, Rome.
- Goode, M.J. & Sasser, M.** 1980. Prevention – the key to controlling bacterial spot and bacterial speck of tomato. *Plant Disease*. 64: 831–834.
- Gupta, A. & Kashyap, P.L.** 2020. Management of seed-borne diseases: An integrated approach. In R. Kumar & A. Gupta, eds. *Seed-borne diseases of agricultural crops: detection, diagnosis & management*. https://doi.org/10.1007/978-981-32-9046-4_25
- Hausbeck, M.K., Bell, J., Medina-Mora, C., Podolsky, R., Fulbright, D.W.** 2000. Effect of bactericides on population sizes and spread of *Clavibacter michiganensis* subsp. *michiganensis* on tomatoes in the greenhouse and on disease development and crop yield in the field. *Phytopathology*. 90(1): 38–44. [Cited 12 August 2022]. <https://apsjournals.apsnet.org/doi/abs/10.1094/PHTO.2000.90.1.38>
- ISPM 27.** 2016. *Diagnostic protocols for regulated pests*. DP7. Potato spindle tuber viroid. Rome. IPPC Secretariat, FAO.
- ISPM 31.** 2008. *Methodologies for sampling of consignments*. Rome. IPPC Secretariat, FAO.
- ISF (International Seed Federation).** 2017. *Method for the Detection of Xanthomonas spp. on Pepper Seed*. Nyon, Switzerland, ISF. 11pp. [Cited 12 August 2022].

https://worldseed.org/wp-content/uploads/2017/07/Pepper_Xanthomonas_spp_July2017.pdf

- ISF.** 2019a. *Detection of Infectious Tobamoviruses in Pepper Seed*. Nyon, Switzerland, ISF. 8pp. [Cited 12 August 2022]. https://worldseed.org/wp-content/uploads/2019/09/Pepper-Tobamo_2019.09.pdf
- ISF.** 2019b. *Detection of Infectious Tomato brown rugose fruit virus (ToBRFV) in Tomato and Pepper Seed*. Nyon, Switzerland, ISF. 11 pp. [Cited 12 August 2022]. https://worldseed.org/wp-content/uploads/2019/09/Tomato-ToBRFV_2019.09.pdf
- Jones, J.B., Pohronezny, K.L., Stall, R.E. & Jones, J.P.** 1986. Survival of *Xanthomonas campestris* pv. *vesicatoria* on tomato crop residue, weeds, seeds, and volunteer tomato plants. *Phytopathology*. 76: 430–434.
- Lee, Jung-Myung.** 2004. Advanced in seed treatments for horticultural crops. *Chronica Horticulturae* 44 (2): 11-20
- Levitzky, N, Smith, E, Lachman, O, Luria, N, Mizrahi, Y, Bakelman, H, Sela, N, Laskar, O, Milrot, E & Dombrovsky, A.** 2019. The bumblebee *Bombus terrestris* carries a primary inoculum of *Tomato brown rugose fruit virus* contributing to disease spread in tomatoes. *PLoS ONE*. 14(1): 1–13.
- Lewis Ivey, L.M.** 2013. Seed treatments: vegetables. The Ohio State University Extension, The Vegetable Seed Treatment Section. 5 pp. [Cited 11 August 2022]. <https://www.lsuagcenter.com/NR/rdonlyres/26772246-4C4A-4028-992D-95BF6DD51C6D/96988/43SeedTreatmentsVegetables2014FINAL.pdf>
- Lewis Ivey, M.L., Nava-Diaz, C. & Miller, S.A.** 2004 Identification and management of *Colletotrichum actiuatum* on immature bell peppers. *Plant Disease*. 88(11): 1198–1204.
- Maloy, O.C.** 2005. Plant disease management. *The plant health instructor*. The American Phytopathological Society DOI: 10.1094/PHI-I-2005-0202-01
- Peusens, C. & Lesprit, E.** 2020. Good seed and plant practices – an example of a process approach to preventing Cmm from entering the tomato propagation chain. *Acta Horticulturae*. 1273. ISHS 2020. DOI 10.17660/ActaHortic.2020.1273.40
- Ritchie, D.F.** 2000. Bacterial spot of pepper and tomato. *The Plant Health Instructor*. doi:10.1094/PHI-I-2000-1027-01. Updated 2007.
- Sheu, Z.-M., Wang, J.-F. & Jackson, G.** 2022. Capsicum (chilli) anthracnose (177). *Pacific Pests, Pathogens & Weeds Fact Sheets*. University of Queensland and the Secretariat of the Pacific Community. [Cited 11 August 2022]. https://apps.lucidcentral.org/ppp_v9/text/web_full/entities/capsicum_chilli_anthracnose_177.htm
- Sijam, K., Chang, C.J. & Gitaitis, R.D.** 1991. An agar medium for the isolation and identification of *Xanthomonas campestris* pv. *vesicatoria* from seed. *Phytopathology*. 81: 831–834.
- Smith, K.L.** 2000. “Peppers,” in *Ohio vegetable production guide*, ed. R.J. Precheur. Columbus, Ohio, Ohio State University Extension. pp. 166–173. [Cited 11 August 2022]. <https://ag.purdue.edu/btny/midwest-vegetable-guide/Pages/default.aspx>
- Verhoeven, J.T.J., Jansen, C.C.C, Willenmen, T.M., Kox, L.F.F., Owens, R.A. & Roenhorst, J.W.** 2004. Natural infections of tomato by *Citrus exocortis* viroid,

Columnnea latent viroid, Potato spindle tuber viroid and Tomato chlorotic dwarf viroid. *European Journal of Plant Pathology*. 110, 823-831.

Waller, J.M. 1992. "Colletotrichum diseases of perennial and other cash crops," in *Colletotrichum: biology, pathology and control*, J.A. Bailey and M.J. Jeger, eds. Wallingford, UK, CAB International. pp. 167–186.

Welideniya, W.A., Rienzie, K.D.R.C., Wickramaarachchi, W.A.R.T. & Aruggoda, A.G.B. 2019. Characterization of fungal pathogens causing anthracnose in capsicum pepper (*Capsicum annuum* L.) and their seed borne nature. *Ceylon Journal of Science*. 48(3): 261–269.

Additional resources

Hausbeck, D.L., Thompson, C.M., Hilgren, J. & Lovic, B. 2003. Wet seed treatment with peroxyacetic acid for the control of bacterial fruit blotch and other seedborne diseases of watermelon. *Plant Disease*. 87: 1495–1499.

IPPC (International Plant Protection Convention) Secretariat. 2020. Specification 70. Annex Design and use of systems approaches for phytosanitary certification of seeds (2018-009) to ISPM 38 (International movement of seeds). Rome, Italy, IPPC Secretariat, FAO. [Cited 12 August 2022]. https://assets.ippc.int/static/media/files/publication/en/2020/12/Spec_70_RevISPM38_En_2020-12-02.pdf

ISF Working Group Vegetable Seed Production & APSA's Vegetable and Ornamentals Special Interest. 2020. Good practices for Healthy Vegetable Seed Production. ISF/APSA. 8 pp.

ISTA (International Seed Testing Association). 2022a. *International rules for seed testing, 2022*. Chapter 2: Sampling. Bassersdorf, Switzerland, ISTA. <https://doi.org/10.15258/istarules.2022.02>

ISTA. 2022b. *International rules for seed testing, 2022*. Chapter 7: Seed Health Testing. Bassersdorf, Switzerland, ISTA. <https://doi.org/10.15258/istarules.2022.07>

Lewis Ivey, M.L. & Miller, S.A. 2003. Evaluation of fungicides and a biocontrol agent for the control of anthracnose on green pepper fruit. Nematicide Test Report [Online], New Fungicide and Nematicide Data Committee of the American Phytopathological Society.

