Canadian Guidelines for the Responsible Conduct of Field or Greenhouse Studies Inoculated with Plant Pathogens

 (Code of Practice)

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# Background

New diseases and changes in existing pathogens represent ongoing threats to agricultural production, forestry, and the environment more broadly. Phytopathological research plays an instrumental role in developing new and innovative ways to manage plant diseases and reduce the impact of pathogens and other pests on yield stability, economic sustainability, consumer safety, and the health and diversity of the environment. The work conducted by plant pathologists in Canada leads to the development of new and improved disease management strategies that reduce the threat of disease epidemics in agricultural production and help to reduce the environmental footprint as a consequence of farm pesticide application.

Research trials involving inoculum production and manual inoculation of plant pathogens have been a standard part of phytopathology research since the mid eighteenth century when, in 1755, Tillet demonstrated that black dust carried by wheat seeds was a factor in the spread of bunt [[reviewed in 1](#_ENREF_1)]. These types of trials are conducted to answer a wide range of research questions and address a broad array of issues, including plant disease epidemiology, development of resistant varieties/cultivars, and evaluation of crop protection products.

Studies under controlled conditions and in laboratories provide important insights into specific components of the disease cycle and the factors that affect infection, symptom development, and epidemiology. While important, studies of this nature have significant limitations since they examine the reaction of individual plants as isolates rather than populations. They also cannot simulate the full range of environmental conditions that plant and pest population will be exposed to under field conditions. To address these limitations, the research will eventually need to move out of the laboratory and into the field.

Field trials present a separate set of challenges. While laboratory trials may be repeated multiple times within a year, field trials in Canada are generally limited to one per year at a particular site. Field research is also inherently more variable than laboratory trials, making results less predictable and repeatable. Researchers cannot change the weather but they can influence factors such as inoculum pressure and infection success by selecting the timing of inoculation and manipulating environmental parameters (e.g., via irrigation or covers). Thus, inoculation can not only increase the probability of achieving a successful result (uniform, useful and reliable data) but also provide information that could not be achieved in any other way.

The majority of plant pathogens in Canada that are being studied in inoculated field trials are known to cause economic harm to agricultural or horticultural crops or forest resources. Knowingly releasing a plant pathogen into the environment should therefore not be done without examining the potential risks associated with this action.

The purpose of this document —hereinafter referred to as “the guidelines” — is to provide best practices for researchers who are conducting field or unconfined greenhouse trials that require inoculation with plant pathogens. The objective is to ensure that the activities surrounding an individual study, as they pertain to inoculum production, transport and pathogen release, are conducted in a responsible manner that take into account any potential for harm that could occur to other living organisms (including humans), to the environment in general, and to the agriculture and agri-food industry.

The intended audiences for these guidelines are professional researchers (public, academic, industry) working with plant pathogens that routinely occur in commercial fields and environments, in unconfined greenhouses or in field trials, as well as the manufacturers of inoculum for research purposes (including those who produce inoculum for third party researchers).

Adherence to the guidelines is expected to benefit the professional plant pathology community by standardizing research practices and avoiding unintended releases of plant pathogens. As many of these measures have been in practice since the early 1990’s, the guidelines are not intended to supplant current best practices but rather to frame established practices into a broadly applicable document. Adopting these measures will contribute to rigorous, consistent, replicable results; minimize potential risks associated with these endeavours to human health and the environment including other crops; and encourage compliance with statutory requirements.

# The Objective of the Guidelines

The contributors listed in [Appendix I](#AppendixI) have developed these guidelines, on behalf of the community of plant pathology researchers in Canada, to codify the best practices that have long existed within this field. The guidelines will be maintained by the Canadian Phytopathological Society (CPS) and made available on the CPS website. The Society will revise them periodically as needed, in consultation with the research community, to ensure they remain current.

These guidelines are intended to be a guide to best practices in the production, distribution, and use of plant pathogens in research in order to minimize or eliminate any potential risks to the environment or human health while at the same time facilitating rigorous, creative, and beneficial research.

Adherence to the guidelines described in this document will help to address concerns regarding potential risks to human health or the environment associated with the manufacture, transport, and redistribution of plant pathogens within the Canadian environment; ensure a high degree of public confidence that the research is being conducted in a safe manner; and ensure consistency in the way that biosafety aspects of plant pathogen research trials are addressed across Canada.

# Scope of the Guidelines

These guidelines apply to the production, storage, transportation, and release into the environment of plant pathogens for the purpose of inoculating field or greenhouse trials by qualified researchers.

These guidelines apply to indigenous, endemic plant pathogens that are, or have been, commonly identified and confirmed in Canada during plant surveys and its occurrence in the region where the inoculation will take place is well documented.

Plant pathogens that fall under the following categories should not be produced en masse or considered for field or unconfined greenhouse release under any circumstances:

1. Plant pathogens not confirmed to occur in Canada:
	1. The pathogen does not occur in Canada or has not been previously documented as occurring within Canada.
2. Isolates of plant pathogens that are present in Canada, but the isolate itself is of foreign origin:
	1. While the pathogen may occur in Canada, the specific isolate or culture of interest is not of Canadian origin.

Adherence to these guidelines does not necessarily exempt researchers from notification requirements or other statutory controls. The following Acts and their associated regulations should be considered prior to initiating any plant pathogen field trials:[[1]](#footnote-1)

* Canadian Environmental Protection Act, 1999
* Pest Control Products Act
* Plant Protection Act
* Fertilizers Act
* Export and Import Permits Act
* Provincial and/or municipal plant protection acts and regulations[[2]](#footnote-2)

Foremost among these statutes is the Canadian Environmental Protection Act, 1999 (CEPA, 1999) [[2](#_ENREF_2)] and the New Substances Notification Regulations (Organisms) (NSNR(O)), which implement Part 6 of CEPA, 1999 and outline notification and information requirements for any person planning to import into Canada or manufacture (produce) a living organism that is new to Canada [[3](#_ENREF_3)]. It is important to note that under this legislation, any living organism that does not reside on the Domestic Substances List is considered “new”. By this definition all plant pathogens are ‘new’ to Canada and therefore subject to the NSNR(O) regulations. Nonetheless, certain microorganisms may be exempt from notification if certain conditions are met.[[3]](#footnote-3)

1. The organism is naturally occurring and has been isolated from the Canadian environment;
2. The organism has been accurately identified;
3. The organism is to be released only in agricultural field trials;
4. Manufacture (scaling-up, production, or culturing) and activities related to the release of the organism at the trial site are conducted in a manner that conforms to a recognized Code of Practice established to minimize dispersal of the organism and any potential impacts to the environment and human health.
5. The quantity manufactured and released at any one time is limited to the minimum quantity required to meet the objectives of the field trial; and
6. The field trial uses the minimum area, consisting of one or more sites as required to meet the objectives of the field study.

Other federal Acts and associated regulations that may apply to the manufacture and application of plant pathogens include the Pest Control Products Act (PCPA), which regulates the use of microorganisms used to control pests [[4](#_ENREF_4)]; the Plant Protection Act and regulations, which prohibit or restrict the movement into, within, and out of Canada of any plant pest [[5](#_ENREF_5)]; the Fertilizers Act, which regulates the use of microorganisms as plant supplements [[6](#_ENREF_6)]; and the Export and Import Permits Act, which regulates any plant pathogens that could be used to develop biological weapons [[7](#_ENREF_7)].

Additional provincial regulations may also cover the use and/or movement of plant pathogens within their jurisdictions. Researchers should consult the appropriate regulatory bodies within their own province prior to commencement of field or greenhouse trials.

# Definitions

**Containment:** Restricting plant pests to their intended locations through the use of operational procedures, physical barriers and facility design [[8](#_ENREF_8)].

**Containment facility:** A structure whose purpose is to prevent escape of material held within it, into the environment [[8](#_ENREF_8)].

**Domestic Substances List (DSL):** An inventory of substances manufactured in, imported into or used in Canada between January 1, 1984 and December 31, 1986. The DSL is the sole standard against which a substance is judged to be "new" to Canada. With few exceptions, all substances not on this list are considered new and must be notified prior to importation or manufacture in order that they can be assessed to determine if they are toxic or could become toxic to the environment or human health.[[4]](#footnote-4)

**Indigenous:** A microbial isolate that is isolated from, or known to occur in, the ecozone(s) of intended use [[9](#_ENREF_9)].

**Manufacture:** Intentional production or culturing of the organism and production of inoculum (including scaling-up, production, or culturing).

**Natural Forests:** Forests with natural species and ecological processes and for which there has been continuity of ecological processes over a very long period of time [[10](#_ENREF_10)].

**Plant Pathogen:** Any organism that is injurious or potentially injurious, whether directly or indirectly, to plants or to products or by-products of plants, and includes any organism prescribed as a pest [[5](#_ENREF_5)]. This includes any species, strain or biotype of pathogenic agent that is injurious to plants or plant products [[11](#_ENREF_11)] including, but not limited to, bacteria, nematodes, fungi and oomycetes, phytoplasmas, viruses and viroids [[8](#_ENREF_8)].

**Researcher:** For the purpose of this document a researcher is any qualified person involved in the planning or implementation of plant pathology trials. This could include program directors, research scientists, and field or greenhouse trial managers.

# Plant Pathogen Risk Assessment

The primary goal of a biosafety risk assessment is to identify and mitigate risk. Risk is a function of hazard and exposure [[12](#_ENREF_12)]. The risk assessment process begins with an evaluation of hazard — in which the intrinsic potential adverse effects of the microorganism on organisms and processes are identified and quantified — and is followed by an exposure assessment in which the potential distribution of the microorganism is estimated. As such, hazard describes the potential for the microorganism to elicit a harmful effect; risk describes the probability of this harmful effect occurring [[reviewed in 13](#_ENREF_13)].

Plant pathogens almost never infect or infest healthy humans; however, they may potentially pose a risk to agricultural production, forests, and natural environments [[8](#_ENREF_8)]. As such, prior to the production or release of inoculum, the researcher should conduct a risk assessment to identify potential adverse effects and, where applicable, establish risk mitigation measures.

Risks associated with specific plant pathogens differ depending on the host species, the biology of the pathogen in question, and the environment. Methods of reproduction and potential for dispersal (e.g., via vectors) should be considered as part of this assessment. It is not practical within these guidelines to assess the risk associated with each plant pathogens for every host species. Rather, these guidelines provide a framework to guide the risk assessment for an individual pathogen.

## Qualifications of the Risk Assessor

The principle investigator and his/her employees are generally the most appropriate individuals to evaluate the risk associated with their research and laboratory practices. The basic qualifications required of these individuals include:

* Knowledge of the hazards associated with the pathogen in question.
* Knowledge of the procedures and techniques employed in the study.
* An understanding of how the procedures and techniques may affect potential risk.
* Knowledge of containment requirements, national and provincial standards and guidelines.

Researchers whose area of expertise lies outside of plant pathology or who lack familiarity with the Canadian agriculture, horticulture, or forestry industries should seek advice from experienced plant pathologists who are members of the Canadian Phytopathological Society and who have worked with the pathogens and plants in question.

## Identification and Classification of the Microorganism

[Appendix II](#AppendixII) includes specific references to aid in the accurate identification of organisms to the level of the species and, if appropriate, to the subspecies (i.e. strain) level. It is not intended to be exhaustive and will be subject to revision at the discretion of the Canadian Phytopathological Society.

The approach used in pathogen identification should be recorded and a rationale for the identification should be documented. If the identification was conducted by a third party laboratory, all relevant documentation pertaining to the identification should be preserved.

For known plant pathogens, it is important to ascertain that the microorganism is indigenous to Canada. As such, the origin of the isolate (including the name of the host plant species and cultivar where possible), the geographic location of sample collection, and the date of sample collection and isolation must be documented. A description of the native habitat and ecological range in Canada should be ascertained and recorded. A specimen should be preserved in the laboratory strain collection or submitted to an independent bioresource centre for long-term storage.

Researchers should conduct, and document, an analysis of the identified microorganism and its history to ensure familiarity with factors that may affect the level of risk that it could represent. These include documentation of previous occurrence and confirmation of identification in Canada; history of safe use in agricultural field trials (if available); analysis of reproductive strategy; host range; mode of transmission or spread in the environment; and potential persistence in the environment.[[5]](#footnote-5) Where available, primary research data on the microorganism in question should be used for this analysis; however, in many cases it will be necessary to rely on the published literature (including the grey literature) for this information.[[6]](#footnote-6)

If the microorganism is identified as a species that has not previously been identified as a plant pathogen, it may require further study and the application of additional risk mitigation measures may be deemed necessary.[[7]](#footnote-7) Considerations for research on these organisms are outside of the scope of this guidance document.

Using the information ascertained above, researchers should evaluate the potential of the microorganism to persist or spread in the environment and, where necessary, identify methods to manage this risk throughout the experimental process. All protocols used, and experimental results derived, should be recorded.

# Inoculum Production and Handling

Protocols for the production of plant pathogen inocula will vary based on the nature of the specific pathogen and the intended method of release. The purpose of the guidelines is not to provide specific methodologies but to ensure that production activities reduce any potential risks identified during the risk assessment process. For guidance on specific methodologies, the reader is directed to a number of existing publications on a wide variety of production and inoculation protocols for different plant pathogens [for example, see [15](#_ENREF_15), [16](#_ENREF_16), [17](#_ENREF_17)].

## Production Site

Facilities producing inocula within the scope of these guidelines do not require certification but, at a minimum, should be able to meet the physical requirements and operational standards of the CFIA’s PPC-1 (Plant Pest Containment Level 1) for common, low-risk organisms [[8](#_ENREF_8)].

Plant pathogen inocula should contain only the test organism and must be free of other microbial contaminants.

Responsibilities of the facility producing the inoculum include:

1. Accurate identification of organism (including documentation of its source)
2. Maintenance of the physical facility
3. Maintenance of and adherence to operational practices that pertain to but are not limited to:
	1. Facility access
	2. Documentation of pest
	3. Training of personnel
	4. Use of appropriate personal protective equipment (PPE) for safe handling of the pathogen (minimum PPC-1 requirements)
	5. Workplace sanitation
	6. Inoculum storage
	7. Inoculum disposal and decontamination
4. Maintenance of records of inoculum production
5. Ensuring that recipients of the inoculum, are also aware of these guidelines

In the event that the producer of the inoculum is not the research trial manager, the trial manager should ensure that the producer is aware of these guidelines.

## Storage and Transport

All inocula should be stored in leak-proof containers. A primary container may be sufficient for temporary storage but it is recommended that inocula intended for long term storage or transport be placed inside a sealed, shatter-proof secondary container.

The primary container should be labelled to clearly identify the contents and storage requirements. This should include, at a minimum, the following information:

* Pathogen identity
* Volume of inoculum and concentration if applicable
* Production date

The secondary container should, at a minimum, be labelled with the name and contact information of the individual and/or research group responsible for the inoculum.

Additional information, such as storage requirements and/or disposal requirements, should be added where appropriate. Specific storage conditions will depend on the environmental requirements of the pathogen and the length of time the inoculum is intended to be stored. Excess inoculum that is not required, or for which long term storage is not available, should be rendered nonviable and the containers either destroyed or disinfested as soon as is practical.

This document does not define a maximum amount (volume or weight) of inoculum that can be produced through manufacture; however, the amounts distributed to trial sites should not exceed those required for use in the trial. Excess inoculum can be stored indefinitely if storage conditions are consistent with current Canadian federal guidelines [[8](#_ENREF_8), [18](#_ENREF_18)].

# Environmental Release/Trial Inoculation

Researchers should document the location of the trial site, size of the research plots, and the timing, rate, and inoculation method of the pathogen to those plots. The life stage of the pathogen to be inoculated and the concentration to be applied should be recorded.[[8]](#footnote-8) The method of application could affect potential dispersal in the environment so the research protocol should include sufficient isolation distances from sensitive areas (including commercial production fields) and any additional operational measures necessary to adequately contain dispersal of the microorganism. Conformity to isolation distances and additional operational measures should be documented and the documents retained.

Unused inoculum should be returned to the laboratory and stored or disposed of as per current federal guidelines [[18](#_ENREF_18)].

## Containment

Although the plant pathogens that fall under these guidelines are already present in the environment in Canada, trial managers have a responsibility to reduce the risk of dispersal beyond the research trial, especially if the microorganism is of limited distribution. Containment measures may be temporary, such as those specific to the inoculation event, others may need to be implemented for the duration of the trial, and some may be required to manage the site after the trial is completed. Specific measures will vary depending on the locale and the risks identified during the pest risk assessment but may include some of the following strategies:

* Restrict inoculation event depending on environmental conditions
* Sanitation requirements for equipment used in inoculation
* Buffer zones to achieve spatial isolation from host crops
* Physical barriers (e.g., non-host crops used as borders around trial site)
* Restricting access to the trial site
* Removal of infected material from site upon termination of trial and proper disposal
* Recropping restrictions for the following growing season
* Cleaning and disinfection (sanitizing) of greenhouse benches and other potentially contaminated surfaces, trays, etc., at the end of the trial.

Researchers should document how containment will be achieved, for example, through the sanitation of field and equipment, cultivation of the soil, crop rotation, and spatial isolation from nearby commercial operations. Researchers are advised to follow the Canadian Food Inspection Agency Basic Containment Standards for facilities handling plant pests throughout the course of the study [[8](#_ENREF_8)].

Researchers should consider the host range of the pathogen and the presence of any susceptible hosts in close proximity to the field site. If potential hosts are identified, strategies to mitigate potential risk should be developed and documented. Examples include (but are not limited to) removal of host species, use of a different application method, or selection of an alternative site.

Field equipment has the potential to harbour and transmit inoculum. Sanitation practices to remove excess soil and debris from field equipment exposed to inoculum should be conducted at the field trial site if possible. These can include brushing off of visible debris, high-pressure washing and, if appropriate, sterilization/bleaching of the equipment. All hand tools and exposed surfaces should be cleaned and, if required, treated with a disinfectant that is effective against the plant pathogen.

## Additional Exposure Considerations

The researcher should ensure and document that all personnel who could be exposed to the microorganism during the course of the study (inoculation, trial maintenance, and trial rating phases) wear the proper personal protective equipment (PPE) for their activity and level of exposure to the plant pathogen. Basic PPE for handling and applying any plant pathogen inoculum would include rubber gloves, eye protection, a lab coat or disposable TYVEC suit, and a particle respirator or dust mask. Depending on the pathogen, personnel involved in crop maintenance or trial rating may not need to wear any protective equipment — in the case of common, low-risk pathogens that are already widely present on the crop and in the environment — or they may need to wear latex gloves, eye protection or a dust mask.

Contingency plans should be developed to deal with accidental releases or spills of inoculum. Specific measures will depend on the type and quantity of inoculum, the risk associated with the pathogen and the location where the release occurred. The researcher should document that appropriate measures were taken.

# Guidelines for use of Microorganisms in Greenhouses

Plant disease trials in commercial greenhouses generally are performed with natural inoculum, i.e., inoculum already present, naturally, in the commercial crop or greenhouse. Plant disease research trials are very rarely inoculated in commercial greenhouses because commercial producers do not want to add to the natural pathogen load already present; are generally reluctant to leave untreated (control) plants; and, as a rule, grow and maintain the crop and environment in such a way as to be as unfavourable to plant disease development as possible.

If an inoculated trial is performed in a commercial greenhouse, the plant pathogenic organism and strain should already be present in the commercial greenhouse (i.e., the plant pathogen should have been isolated from the trial greenhouse) and the grower should be made aware of what is being introduced and how it will be managed. The researcher and grower should agree on what steps will be taken to limit spread of the disease in the greenhouse and how the pathogen and the diseased crop will be removed and destroyed at the end of the trial, and the area cleaned and disinfected.

Inoculated plant disease trials are commonly conducted in designated research greenhouses, at educational institutions, government research facilities, or private companies. The same recommendations for field trials, above, apply to uncontained research greenhouses where the pathogen may move into the surrounding environment on air or water:

* Only local pathogen strains should be used;
* Entry to the trial area should be restricted;
* Barriers should be erected, where possible, to prevent movement of inoculated pathogens to other, nearby research bays or houses;
* Infected plant material must be removed and properly disposed of at the end of the trial; and
* Greenhouse benches and other structures (e.g., plastic or glass walls, ground cloth, or capillary mats) must be decontaminated at the end of the trial.

In terms of PPE, the researcher should consider that the concentration of airborne plant pathogen spores may be higher in a contained space, such as a greenhouse, than in a field situation, and thus, a higher level of PPE may be needed for personnel applying inoculum or spending a significant amount of time working in the inoculated crop, either doing crop maintenance or trial rating.

Entry to the greenhouse trial should be controlled and restricted, as much as possible.

Greenhouses are usually vented to the outdoors and often have fans that may move airborne pathogens and drainage systems that can move water-borne pathogens. Local strains of common plant pathogens will already be present in the natural environment surrounding the greenhouse, but the researcher should consider where the inoculated strain may enter the outdoor environment and any potential effect on nearby crops or other research trials.

# Appendix I. Contributors

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# Appendix II: Identification and Taxonomic Classification of Microorganisms

Accurate identification and taxonomic classification of a microorganism is imperative in differentiating it from isolates that are detrimental to human health or the environment. As such, adherence to these guidelines requires a minimum of species-level identification. Where appropriate, identification to the strain level may be necessary; for example, to distinguish between closely related microorganisms with differing risk profiles. However, many environmental isolates have never been characterized in detail, therefore identification to the strain level would be challenging in many cases.

The choice of appropriate method for identification is at the discretion of the field trial manager. This appendix is intended to provide a high-level summary of the various tools available for identification; researchers are encouraged to consider the strengths and weaknesses of each approach and to adopt methods that result in a conclusive and definitive identification of the microorganism and that allow for clear differentiation of the organism from any closely related pathogenic and/or toxigenic species or (where appropriate) strains.

Researchers should ensure that the method used is accurately recorded and that the data to support the identification are readily available.

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| **Approaches** |  | **Examples** | **Representative references** |
| Molecular/Genotypic Approaches | Genotypic | 16S rRNA sequencing | [[19](#_ENREF_19)] |
| Ribotyping | [[20](#_ENREF_20)] |
| rep-PCR (DNA barcode) | [[21](#_ENREF_21), [22](#_ENREF_22)] |
| Internal Transcribed Spacer (ITS) sequencing | [[reviewed in 23](#_ENREF_23)] |
| Proteomic | MALDI-TOF | [[24](#_ENREF_24), [25](#_ENREF_25)] |
| Vibrational spectroscopy  | [[26](#_ENREF_26)] |
| Serological identification, e.g., Enzyme-Linked Immunosorbent Assay (ELISA) | [[reviewed in 27](#_ENREF_27)] |
| Traditional Approaches | Morphological and phenotypic characterization | For example, colony or spore shape; size; sporulation characteristics; cell surface and cell wall (e.g., Gram reaction) characteristics; motility; etc. | [[28](#_ENREF_28), [29](#_ENREF_29)] |
| Fatty acid characterization |  | [[30](#_ENREF_30), [31](#_ENREF_31)] |
| Biochemical analysis | Growth requirements, enzymatic profile, and cellular fatty acid composition. | [[29](#_ENREF_29)] |

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1. This list was complete at time of drafting; however, other federal Acts and regulations may also apply. [↑](#footnote-ref-1)
2. A comprehensive list of these is outside of the scope of these guidelines. Researchers are advised to consult with the appropriate regional regulatory authorities. [↑](#footnote-ref-2)
3. Note that at the time of drafting, these amendments were being considered but had not yet been approved. Advice on current legislative provisions can be obtained from Environment Canada: <http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=E621534F-1>. [↑](#footnote-ref-3)
4. A current list of all organisms listed on the DSL may be found here: <http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=C4E09AE7-1> [↑](#footnote-ref-4)
5. Of note, the persistence of an unmodified microorganism in its natural environment is largely predictable [[14](#_ENREF_14)]. [↑](#footnote-ref-5)
6. Given the low risk profile of these organisms, it is conceivable that a lot of existing research would not be published in the peer-reviewed literature. Grey literature sources — which include theses, patents, technical reports from government agencies or scientific groups, white papers, and conference reports — may provide more appropriate sources. [↑](#footnote-ref-6)
7. The application of Koch’s Postulates may be appropriate; however, discussion of methodologies to characterize new pathogen species this is beyond the scope of these guidelines. [↑](#footnote-ref-7)
8. For example, number of conidia per ml or weight of dried sclerotia per gram of grain [↑](#footnote-ref-8)
9. Current affiliation: Southwest Florida Research and Education Center. [↑](#footnote-ref-9)