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CANADA UNITED STATES MEXICO

NAPPO Regional Standards for Phytosanitary Measures (RSPM)

RSPM No. 27
Guidelines for Importation and Confined Field Release of Transgenic Arthropods in NAPPO Member Countries

The Secretariat of the North American Plant Protection Organization
1431 Merivale Road, 3rd Floor, Room 309
Ottawa K1A 0Y9 Canada
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Review

NAPPO Standards for Phytosanitary Measures are subject to periodic review and amendment.

The next review date for this NAPPO Standard is 2012. A review of any NAPPO Standard may be initiated at any time upon the request of a NAPPO member country.

Endorsement

This Standard was approved by the North American Plant Protection Organization (NAPPO) Executive Committee on October 22, 2007.

Signed by:

Greg Stubbings
Executive Committee Member
Canada

Paul R. Eggert
Executive Committee Member
United States

Javier Trujillo Arriaga
Executive Committee Member
Mexico

Implementation

See the attached Implementation Plans for implementation dates in each NAPPO country.

Amendment Record

Amendments to this Standard will be dated and filed with the NAPPO Secretariat. The most recent version will be posted on the NAPPO website at: www.nappo.org/stds_e.htm.

Distribution

This Standard is distributed by the Secretariat of the NAPPO within NAPPO, including Sustaining Associate Members and Industry Advisory Groups, to the FAO IPPC Secretariat, to the ICGPP, and to the Administrative Heads of the Regional Plant Protection Organizations (RPPOs).

Introduction

Scope

This Standard is designed to provide guidance to NAPPO member countries on the importation and confined field release of transgenic arthropods that are known plant pests or have the potential to affect plant health. This includes transgenic arthropods used for biological control and transgenic beneficial arthropods with the potential to affect plant health. Transgenic arthropod species that are not plant pests, but that may pose a phytosanitary risk because of the genetic modification may also be considered under this Standard.

Transgenic arthropods that have undergone a previous pest risk analysis by the regulatory authority of the importing country and been determined not to pose a phytosanitary risk may be exempted from importation requirements under this standard. Paratransgenic arthropods, nematodes, and other animals that may pose a risk to plants are specifically excluded from the scope of this Standard, although they may be considered at a later time.

Issues relating to the potential adverse impact of transgenic arthropods on human and animal health or on biological diversity and the environment beyond direct and indirect impacts on plant health are not relevant to plant pest issues and fall outside the scope of this NAPPO Standard. Authorization for importation and/or release of transgenic arthropods may also depend on additional analyses performed by other regulatory authorities in the NAPPO member country to evaluate environmental and/or human health impacts.

Note that “confined field release” includes not only physical confinement, such as caged releases, but also releases where establishment and spread of the transgenic arthropods is restricted by biological, temporal, or geographic mechanisms. Guidance for unconfined release of transgenic arthropods into the environment is not provided in this Standard. It is anticipated that this issue will be addressed as the NAPPO member countries gain additional experience in regulating transgenic arthropods.

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Definitions, Abbreviations, and Acronyms[1]

Definitions of phytosanitary terms used in the present standard can be found in ISPM 5 and RSPM 5.

Outline of Requirements

Authorization for the importation and confined field release of transgenic arthropods that have implications for plant health may require that:

- The applicant provides sufficient information to identify the transgenic arthropod, the type of action proposed, and the proposed and available risk management options.
- The NPPO conducts a pest risk analysis (PRA) of the phytosanitary risk potential associated with the unmodified recipient arthropod and the transgenic arthropod.
- The NPPO determines that specific PRA criteria and any risk management options intended to minimize potential phytosanitary risk associated with the transgenic arthropod have been met.

Background

The technology for development of transgenic arthropods is well established and transgenic arthropod strains are available in laboratories for evaluation and potential future use in some plant pest control programs conducted in NAPPO member countries.

NAPPO member countries should implement authorization systems that provide procedures to assess the phytosanitary risk posed by transgenic arthropods and determine risk management options. A framework for information sharing among NAPPO member countries regarding applications for approvals or permits and actions taken may also be considered. Bilateral workplans could be developed to formalize this framework.

This standard is intended to provide guidance to NAPPO member countries in the use of transgenic arthropods while protecting plant health. Transgenic arthropods released in one country may spread naturally across shared borders, thus impacting other NAPPO member countries. This standard will, therefore address the importation and confined field releases of transgenic arthropods that have implications for plant health.

Applications

A wide range of uses have been proposed for transgenic arthropods that may have implications for plant health. Applications for particular species may differ considerably in scale, the nature of the introduced transgenic trait(s), potential for interaction with the environment, and other aspects that may affect phytosanitary risk. Some examples of actual or proposed applications include:

[1] All definitions cited as (FAO 2007) are from ISPM no. 5, the FAO Glossary of Phytosanitary Terms. Those cited as (FAO 2002) are from the Glossary of Biotechnology for Food and Agriculture - see References section for additional details.

- Fundamental laboratory research conducted in containment facilities, often with the long-term goal of improving plant health.
- Fundamental ecological research conducted under confined field conditions to improve understanding of interactions of the transgenic arthropod with the environment and ecosystems.
- Improvement of mass-reared arthropod strains for plant pest control programs such as the Sterile Insect Technique, e.g., incorporating a genetic marker, a genetic sexing system, a genetic sterilization system, and/or a genetic containment system (Robinson & Franz, 2000; Gong *et al*. 2005).
- Trait improvements for beneficial arthropods, e.g., honey bees or biological control agents (Robinson *et al*. 2000).
- Transgenic arthropods as protein factories to produce specific protein products, e.g., pharmaceutical or industrial applications (Tomita *et al*. 2003).
- Alteration of the sensitivity of a wild pest population to specific environmental conditions, e.g., the presence of a specific pesticide, pro-pesticide, disease, day length, or temperature.
- Alteration of the behaviour or host preference of a wild plant pest population.

Technology

Transposon-based transgenic arthropod technology was originally developed in *Drosophila melanogaster* (Spradling & Rubin, 1982) and has since been used extensively in research laboratories. Transformation using other methods, such as viral vectors or homologous or non-homologous recombination, has also been proposed, however most current transgenic arthropod strains have been developed using transposon-mediated methods (Handler 2001). Transposable elements facilitate integration into host chromosomal DNA because these elements are inherently mobile. Typically, one part of the gene transfer system, the vector, is constructed by removing or disrupting the transposase-encoding region of the transposable element and replacing it with a gene construct that includes a selectable marker gene.

The second part, the transposase source, is typically a helper plasmid containing the transposase-encoding region of the transposon which is required for mobility of the vector. The transposase is often put under heat-shock promoter control, and at least one of the terminal repeats of the helper plasmid is deleted. Therefore, the helper mediates integration of the vector by producing the transposase, but cannot itself integrate into chromosomal DNA. Helper plasmid sequences, and therefore, the transposase protein, will be absent from the transformed strain and the stability of the integrated vector is greatly enhanced. In practice, the vector and helper plasmids are co-injected into pre-blastoderm embryos prior to germ cell formation. The plasmids are enveloped during cell formation, allowing gene transfer to proceed in the nucleus leading, if successful, to the production of a germline mosaic. Injected embryos are reared to adulthood and backcrossed to the host strain, and their progeny screened for marker gene expression.

Currently, there are at least four transposable elements that are used to generate transgenic non-drosophilid arthropods [*Hermes*, *Mos1* (mariner), *Minos* and *piggyBac*]

(Handler 2001). These transposon-based transformation systems have broad functionality, with at least 15 species from 4 different insect orders transformed using the *piggyBac* transposable element (Horn & Handler, 2005). The phenotypic and genotypic characteristics of transgenic arthropod strains may be influenced by 1) the random nature of the gene integrations; 2) the possibility of achieving multiple integrations in a single strain; 3) the effect of the integration on surrounding genes (e.g., position effects); and 4) the potential presence of closely-related transposable elements in the host genome (Handler & McCombs, 2000; Handler *et al*. 2004).

Pest Risk Analysis

The rapid advances in genetic modification of arthropods, including important agricultural pests and beneficial arthropods is accompanied by the need for NPPOs and other regulatory officials to make decisions regarding the use of these organisms. These decisions will include consideration of the potential impacts of transgenic arthropods on plant health. Regulatory decisions on the importation and confined field release into the environment of transgenic arthropods should be science-based and made on a case-by-case basis. Risk management measures should be based on the level of phytosanitary risk. Some of the criteria for risk analysis to be considered may include, but are not limited to (IAEA, 2006):

- Attributes of the unmodified recipient arthropod.
- Ecological relationships and roles of the arthropod species under consideration.
- Attributes of the genetic alteration.
- Phenotype of the transgenic arthropod compared to the recipient arthropod.
- Attributes of the affected environment.
- Likelihood of establishment of the transgenic arthropod in the environment beyond the parameters of the confined field release.

NAPPO member countries have considerable experience with the importation and environmental release of transgenic plants under confined conditions as delineated in RSPM No.14, *Importation and Release (into the Environment) of Transgenic Plants, in NAPPO Member Countries*. Similarly, NAPPO RSPM No.22 *Guidelines for Construction and Operation of a Containment Facility for Insects and Mites used as Biological Control Agents,* provides guidelines for the construction and operation of containment facilities for arthropods used as biological control agents. ISPM No.11 *Pest Risk Analysis for Quarantine Pests, Including Analysis of Environmental Risks and Living Modified Organisms* deals with pest risk analysis of quarantine pests and includes evaluation of environmental risk and risks associated with living modified organisms. Similarly, ISPM No. 3 *Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms,* deals with biological control agents, although this standard specifically excludes transgenic organisms from its scope. These and other NAPPO and ISPM standards have been taken into consideration in the development of this Standard.

1. Importation of Transgenic Arthropods into Containment Facilities

NAPPO member countries should implement authorization systems for the importation of transgenic arthropods that provide procedures to assess the phytosanitary risk posed by the importation and to make decisions on requirements for movement and containment facilities such that unauthorized dissemination into the environment is prevented. Authorization systems should allow for a determination that proposed phytosanitary measures provide conditions under which the transgenic arthropod poses no significant phytosanitary risk. This includes, but is not limited to, adequate genetic characterization of the transgenic arthropod, an acceptable level of security provided by the containment facility, and adequate restrictions on the dissemination of the transgenic arthropod into the environment. The PRA of potential phytosanitary risk associated with the importation of transgenic arthropods for uses other than in containment facilities may require additional information.

Transgenic arthropods may be imported into containment facilities for a variety of reasons. Initial importation of transgenic arthropod strains for any purpose should be into containment facilities, including transgenic arthropods imported for confined field release. Long-term rearing in containment facilities may occur for research, commercial, or other purposes; these activities are not subject to this Standard. Transgenic arthropods that have undergone a previous PRA by the regulatory authority of the importing country and been determined not to pose a phytosanitary risk may be exempted from importation requirements under this standard.

1.1 Information Requirements

Information provided by the applicant should address the administrative information, characterize the transgenic arthropod, and indicate, when applicable, that acceptable containment measures will be taken to prevent dissemination into the environment during transit and while in the containment facility.

1.1.1 Administrative information

The following information should be provided:

- Name, affiliation.
- Complete address, telephone number, and e-mail address of the importer and the exporter.
- Proposed date(s) or period of the importation.
- Quantity being imported.
- Means of transportation and place of entry into the importing country.
- Country or place of origin of the transgenic arthropod. List of any previous authorizations for importation and/or movement of the transgenic arthropod strain.
- Intended use of the transgenic arthropod strain.
- Description of the containment facility and standard operating procedures.
- Signature of the applicant (must be a resident of the country into which the transgenic arthropod strain will be imported) and the date of application.

1.1.2 Description of the transgenic arthropod

1.1.2.1 Description of the recipient arthropod

- Scientific name, synonyms and common names of the arthropod species to which the transgenic arthropod belongs.
- Information on the specific laboratory line or colony of the recipient arthropod, including geographic origin, history of rearing, and history of use in control programs.
- Information on the current and recent historical distribution of the arthropod species in nature.

1.1.2.2 Description of the transgenic technology

- Method of transformation and identification of the transformation vector.

1.1.2.3 Description of the genetic construct

- Name of the genetic construct or plasmid contained in the transgenic arthropod.
- A detailed map and/or description of the genetic construct and list of the genes it contains. This includes marker genes, associated regulatory sequences, and the donor organisms which are the source of the genes. A citation should be provided for each genetic component, if available. Publicly available database citations are acceptable[2].

1.1.2.4 Description of the transgenic arthropod

- Life stage to be imported, e.g., eggs, larvae, pupae, adults.
- Description of the phenotypic expression of the introduced transgenic trait(s) in the transgenic arthropod.
- A description of the molecular, morphological, and/or other methods that can be used to distinguish the transgenic arthropod from the unmodified recipient arthropod.

1.1.3 Risk management options

Guidance documents on acceptable containment of arthropods and risk management options are available (e.g., ASTMH, 2001; NIH, 2002; CFIA, 2005; Public Health Agency of Canada, 2004; RSPM No. 22; ISPM No. 3). The applicant should provide information related to risk management options including:

- Identification, packaging, and segregation measures that prevent and/or minimize mixing, spillage, and dissemination of transgenic arthropod during transit and within and outside the containment facility, except for the purposes of controlled rearing within the containment facility.
- Containment for storage of transgenic arthropods, including all life stages and reproductive cells.
- Monitoring tools (e.g., molecular analysis, phenotypic identification, and trapping) to detect and identify escaped transgenic arthropods in the event of accidental release.

[2] Four commonly used databases and their website addresses are:
DNA Data Bank of Japan: http://www.ddbj.nig.ac.jp/fromddbj-e.html
EMBL Nucleotide Sequence: http://www.ebi.ac.uk/embl/
GenBank: http://www.ncbi.nlm.nih.gov/Genbank/index.html
SWISS-PROT Protein Sequence Data Bank: http://www.ebi.ac.uk/swissprot/index.html

- The applicant should demonstrate that a program is in place that provides ongoing training in the implementation of relevant national, local, and institutional biosafety regulations. The applicant should document that all persons associated with executing the proposed actions have received this biosafety training.
- Contingency plans and risk management measures to be executed in the event of an accidental release of the transgenic arthropod during transport or from the containment facility.
- Devitalization protocols for transgenic arthropods and rearing media when it is no longer in use or authorized. Means of devitalization may include, but are not limited to, dry heat, steam heat, freezing, and/or chemical treatment.
- Disposal protocols for transgenic arthropods and rearing media following devitalization.

1.2 Analysis Criteria

The information required in Section 1.1, and appropriate methods as described in the relevant international standards, provide the basis for an analysis of potential phytosanitary risk associated with the importation of the transgenic arthropod into a containment facility. The regulatory authority in each NAPPO member country should review the information submitted on a case-by-case basis for its completeness and acceptability. When applicable, this may be done by, or in conjunction with, an expert advisory group.

Containment facilities may require an official inspection by the respective NPPO or relevant regulatory authority to determine whether the containment facility is appropriately constructed and managed to limit dissemination of viable transgenic arthropods. Approval of the containment facility may be dependent upon prior official inspections, current facility or equipment certifications, and/or the nature of the transgenic arthropod.

1.3 Authorizations

Authorization to move or import transgenic arthropods should generally be granted when a determination can be made as per Section 1.2 that the proposed importation and containment of the transgenic arthropod does not pose a significant phytosanitary risk.

Authorizations should be conditional on the sole use of the transgenic arthropods at the specific location stipulated on the application. Appropriate means of segregation, containment, and disposal of the transgenic arthropods should be in place to prevent unauthorized mixing, escape and dissemination of the transgenic arthropods. Authorization may be conditional on official inspections to ensure regulatory compliance. Authorization should be conditional on clear identification and containment of the transgenic arthropods during transit and in the receiving facility. When applicable, authorized approvals or permits should be valid for a fixed date or period of time from the date of issue.

1.3.1 Information

The applicant should be responsible for prompt reporting to the regulatory authority of any information relating to deviations from the conditions of the approval or permit and significant changes leading to an increased phytosanitary risk posed by the transgenic arthropod. Accidental releases during shipping or from the containment facility must be reported promptly to the appropriate regulatory authority(ies). The regulatory authority should consider reports of new information or reports of accidental release, and where appropriate, ensure that corrective action delineated in the contingency plan is taken.

1.3.2 Inspection

Upon entry into the importing country, all transgenic arthropods should be subject to inspection or audit according to the specific approval or permit instructions.

1.3.3 Disposal

Consignments of transgenic arthropods not meeting regulatory requirements and/or conditions of entry, should be either confiscated and destroyed or ordered removed from the country into which it is imported, at the importer's expense. Internal packaging should be opened only within the designated containment facility.

1.3.4 Record keeping

Records of imports should be maintained by the importer and should be made available to regulatory authorities upon request.

2. Confined Field Release of Transgenic Arthropods

Transgenic arthropods are reared and/or evaluated in confined field release outside of a containment facility for a variety of reasons. These include, but are not limited to, the evaluation and effect of large-scale production parameters on the genotypic and phenotypic characteristics of the transgenic arthropod, the effect of the introduced transgenic trait(s) on the reproductive ability and survival of the transgenic arthropod as compared to the recipient or wild type arthropod, the effectiveness of the introduced transgenic trait(s) in the field tests, the generation of data required for unconfined field release into the environment, and/or the use of confined conditions for production of commercial products from transgenic arthropods. Confined field release is equivalent to the term experimental release as used in the Mexico biosafety regulations (Mexico, 2005).

Confined field release is intended to minimize interaction between the adjacent environment and the transgenic arthropod, its progeny, products, and the inserted genetic construct. Appropriate confinement conditions may consist of any one or a combination of the following confinement measures:

- physical,
- biological,
- temporal, and/or
- geographic

Decisions to allow a confined field release will be made on a case-by-case basis that considers the information outlined in Section 2.1 below. Information provided should be sufficient in quality and scope to allow for a determination that:

- The transgenic arthropod is adequately characterized.
- An acceptable level of security for movement of the transgenic arthropod from the containment facility to the confined field release site is available.
- The conditions of the confined field release prevent and /or minimize mixing, spillage, and dissemination, and the establishment of viable transgenic arthropods in the environment.
- No viable transgenic arthropods will persist in the environment.
- Unintentional or unanticipated effects, if any, should be restricted to the confined field release site.
- Risk management options (e.g., devitalization, disposal, and detection survey) are in place to adequately limit phytosanitary risk after the confined field release is terminated.

2.1 Information Requirements

Information provided by the applicant should describe the proposed action, characterize the transgenic arthropods, and describe relevant details about the confined field release site. In addition, the applicant should demonstrate that acceptable risk management options have been and/or will be taken to confine the transgenic arthropod to the field site during its release and to prevent persistence of the transgenic arthropod or its progeny in the environment after completion of the confined field release.

2.1.1 Administrative information

The following information should be provided:

- Name, complete address, telephone number, and e-mail address of the person and/or agency/body responsible for the proposed confined field release and name of the person who will be responsible for conducting the confined field release.
- List any previous relevant confined field releases of transgenic arthropod strain(s) of the same species.
- The purpose of the confined field release and proposed experimental design.
- The proposed dates or period of the confined field release(s) and post-release surveillance activities.
- The quantity and life stage of transgenic, recipient, and/or other arthropods being released.
- The number, size, and location of the confined field release site.
- The names and contact information of local, state and federal government agencies that have been notified of the development of the transgenic arthropod and the purpose of the notification (e.g., importation, confined field release).
- The signature of the applicant and the date of application (must be a resident of the country where the confined field release will be conducted).

2.1.2 Description of the transgenic arthropod

Knowledge of the source, function, inheritance, and expression of the transgene in an arthropod strain provides information to assess the potential impact on plant health. The information may be based on data collected from laboratory trials, previous confined field releases, and/or the scientific literature.

2.1.2.1 Description of the recipient arthropod

Information delineated in Section 1.1.2.1, as well as the following information, should be provided, when applicable:

- Plant pest status of the recipient arthropod.
- Distribution and locations, if any, where the arthropod species is known to occur as a plant pest or invasive species.
- Propensity for long distance movement, e.g., long distance flight capacity, wind distribution, or passive transport.
- Life history parameters with emphasis on reproductive biology, hybridization potential with related species, and ability to persist in a viable state under adverse environmental conditions, e.g., diapause.
- The considerations of this section may be fulfilled completely or in part by reference to appropriate scientific and technical literature.

2.1.2.2 Description of the transgenic technology

The following information should be provided, when applicable:

- Description of the transformation method and name of the transformation vector.
  - Indicate pertinent prior use of the transformation vector, or related vectors, to generate transgenic arthropods.
- Detailed map and description of the transformation vector.
  - Indicate use of a pathogenic organism or nucleic acid sequences from a plant pathogen, e.g., viral vector.
  - Indicate use of helper plasmids or a mixture of plasmids. These should be described in detail.
  - Indicate use of site-specific insertion mechanisms, e.g., recombination sites (Horn & Handler, 2005; Siegal & Hartl, 1996).
  - Indicate the use of any transformation stabilization mechanisms (Handler *et al*. 2004, Dafa'alla *et al*. 2006).
- Data on the presence/absence in the genome of the recipient arthropod strain of transposons or other genetic elements closely related to the transformation vector.

2.1.2.3 Description of the genetic construct

The following information should be provided, when applicable:

- Name of the genetic construct contained in the transgenic arthropod.
- Description of the genetic construct contained in the transgenic arthropod.
  - List of the genetic material conferring the desired traits, including marker genes, associated regulatory sequences driving their expression in the arthropod, and gene products.

- A detailed map or description of the genetic construct should be provided, with the location of each genetic component. The map and description should contain sufficient detail to be used in the analysis of data supporting the characterization of the DNA, including, as appropriate, the location of restriction sites and regions used as probes and/or primers for PCR.

- The following information should be provided for each component of the genetic construct:
  - A description of the isolation and characterization of all functional sequences. Publicly available database citations are acceptable. DNA sequence information may be requested by the NPPO.
  - The portion of the functional sequence of the genetic construct from which each genetic component was derived, as designated by relative base pair position or restriction fragments, and its size.
  - The location, order, and orientation in the genetic construct of the genetic material inserted into the recipient arthropod.
  - The intended function of the component in the recipient arthropod.
    - The scientific, common, and/or trade name, of the donor organism.
    - Whether the genetic component is responsible for disease or injury (direct or indirect) to plants and whether it encodes a known toxicant, pathogenicity factor, or irritant.
    - Whether the donor organism is known to directly or indirectly affect plant health.
    - Whether there is a history of safe use of the donor organism or components thereof.

2.1.2.4 Characterization of the transgene inserted into the transgenic arthropod

The following information should be provided, when applicable:

- Description of the molecular and morphological methods that can be used to distinguish the transgenic arthropod from the unmodified recipient arthropod.
  - DNA characterization data may be presented as Southern blot analyses, DNA sequence information, PCR analyses, or other appropriate information.
- Description of the number of transgene inserts, their location in the genome, and methods used to determine this information, e.g., *in situ* hybridization to polytene chromosomes or classical genetic mapping.
- Inheritance pattern of the transgene(s), e.g., homozygous vs. heterozygous, dominant, recessive, sex-linked, autosomal; and the methods used to determine this information.
- Data that demonstrates whether complete or partial copies of coding regions are inserted into the transgenic arthropod genome.
- Data regarding the genotypic and phenotypic stability of the genetic construct in the transgenic arthropod.
- Description of the analytical methodologies used in generating any submitted data, including quality control and quality assurance procedures. Methodologies may be described by reference to citations in the scientific literature.

2.1.2.5 Description of the phenotype of the transgenic arthropod

Information delineated in Section 1.1.2.4 and the following information should be provided:

- Information regarding the phenotypic stability of the introduced transgenic trait in the transgenic arthropod.
- Data on any characteristics of the transgenic arthropod likely to increase plant health risk relative to the recipient and/or wild type arthropod. Such properties may be anticipated due to the nature of the genetic modification or may have been observed during previous rearing or experimental history of the transgenic arthropod. Characteristics that may, if modified, have a potential to alter plant health risk may include, but are not limited to:
    - Life history parameters including the timing and duration of reproduction, number of days until sexual maturity, fecundity, longevity, and survivorship of each life stage.
    - Dispersal ability.
    - Mating competitiveness.
    - Oviposition behaviour.
    - Ability to persist in the environment.
    - Response to specific biotic and abiotic stresses relative to responses of the unmodified arthropod.
    - Response to detection survey tools, e.g., traps, lures, light.
    - Status of susceptibility to labelled insecticide(s) employed as a risk management option or for control of the wild type arthropod.

2.1.3 Confined field release site details

2.1.3.1 Description of the confined field release site

A full description of the geographic landscape of the confined field release site should be provided, including the distance from urban, environmentally-sensitive, and/or other protected areas. The following information should be provided, when applicable:

- A map of the confined field release site, buffer zones, and relevant adjacent areas. Global Positioning System coordinates should be included.
- Relevant details about the confined field release site and adjacent areas. These may include, but are not limited to,
    - Proximity to populations of the same species as the transgenic arthropod and closely related species.
    - Proximity to sensitive or protected ecological areas.
    - Presence of susceptible hosts.
    - Presence of non-target organisms, beneficial arthropods, and endangered or threatened species in the confined field release site. This should take into account the seasonal presence of these organisms, particularly at times of migration and mating.
    - Presence of aboriginal populations of the arthropod or closely related species that may be centers of genetic diversity.
- Description of buffer zones.
    - Presence of potentially-affected non-target organisms, beneficial arthropods, and endangered or threatened species in confined field release site buffer zones.
    - Protocols for surveillance for the presence of transgenic arthropods in the buffer zone.

- Information and test data relevant to identifying the phytosanitary risk including, but not limited to, wild populations of the recipient arthropod species and closely-related species.

2.1.3.2 Description of the confinement measures

- A description should be provided of confinement measures that will be applied to maximize reproductive isolation of the transgenic arthropods from arthropods of the same species that are not part of the confined field release, and to prevent establishment and spread of the transgenic arthropod and interaction with the surrounding environment. Depending on the arthropod species, these measures could include the use of one or a combination of the following methods:
    - Physical confinement such as the use of arthropod proof caging with a double entry mechanism.
        - This should include a description of physical security, access controls, personal protective equipment to be used, and other security measures.
    - Biological confinement such as the release of sterile transgenic arthropods.
        - This should include a description of the biological confinement measures used and data should be provided demonstrating the efficacy of these measures, e.g., the efficacy of genetic- or irradiation-induced sterility
    - Temporal isolation
        - This should include a description of the timing of the release and how it is temporally isolated from sexually-compatible species, host plant use, and/or other intra- and inter-specific interactions that are of concern. For example, release of the transgenic arthropods could be timed to occur before the wild relatives of that species emerge from diapause or after they have begun to enter diapause.
    - Geographic isolation
        - Release of the organism outside their natural habitat or in an area where the organism could not over-winter;

Depending on the arthropod species and designated confinement measures, additional experimental parameters may include:

- Site monitoring for timely removal and disposal of sexually-compatible species.
- Designation of buffer zones adjacent to the confined field release site
    - Use of pesticides on surrounding vegetation.
    - Sterile arthropod releases in buffer zones.
    - Mass-trapping of the wild recipient arthropod in buffer zones.
- Surveillance in the area of the field release and in buffer zones with appropriate tools to detect the presence of the same arthropod species, related species, and for proximity to populations of the same species.
- The experimental design and release protocols including any detection survey of the transgenic arthropods and use of pesticides.

2.1.3.3 Description of the release protocols

The experimental design and release protocols should be provided for confined field release of transgenic arthropods. This includes transport of immature and adult stages to

the release site, method of release, and surveillance activities. Emphasis should be placed on how the protocols compare to, or differ from, protocols previously used for the unmodified recipient arthropod and/or other transgenic arthropod strains of the same species.

2.1.4 Risk management options

For confined field releases, technically justified measures should be taken to confine the transgenic arthropod to the field site during the defined period of release and to prevent the transgenic arthropods or their progeny from persisting in the environment either within or outside of the confined field release site (USDA-APHIS, 2001). These measures should be approved by the NPPO prior to the release of the transgenic arthropod at the confined field release site. Measures may be required after the defined period of release to prevent the transgenic arthropods or their progeny from persisting in the environment either within or outside of the confined field release site. Mitigation measures to address the accidental release should be identified prior to the release of the transgenic arthropod.

2.1.4.1 Risk management measures employed during the confined field release

The following information should be provided, as applicable for consideration on a case-by-case basis:

- Site selection criteria that take into account the potential for interaction with wild populations of the arthropod species and endangered species, adverse weather conditions, flood susceptibility, wind damage to confinement structures, and breaches in security, among other potentially adverse situations during the confined field release.
- Technically justified methods to prevent contact and dissemination of viable transgenic arthropods at the confined field release site by foraging animals, birds, vermin, etc., as warranted.
- Technically justified methods for cleaning of equipment at the confined field release site prior to removal to another location to prevent dissemination of transgenic arthropods into the environment, as warranted.
- Detection methods should be available to distinguish the transgenic arthropods from unmodified organisms, e.g., external fluorescent dyes or internal fluorescent proteins.
- Use and disposal of appropriate personal protective equipment.
- Post-release surveillance of the site and buffer zones for an appropriate period, e.g., one life cycle, after the confined field release is concluded.
- The applicant should demonstrate that a program is in place that provides ongoing training in the implementation of relevant national, local, and institutional biosafety regulations. The applicant should document that all persons associated with executing the proposed actions have received this biosafety training.

2.1.4.2 Handling, disposal, record keeping and other considerations

The following information should be included, as applicable for consideration on a case-by-case basis:

- Means of transportation of transgenic arthropods between the containment facility and the confined field release site and vice versa.

- Technically justified identification, packaging and segregation measures to prevent unauthorized mixing, spillage, and dissemination of transgenic arthropods during transit between the containment facility and the confined field release site.
- Devitalization of surplus transgenic arthropods and any transgenic arthropods remaining after release at the confined field release site by technically justified means including, but are not limited to, dry heat, steam heat, freezing, and/or chemical treatment.
- Disposal protocols for transgenic arthropods and plant material from the confined field release site.
- Transgenic arthropods from the confined field release site should be retained in an NPPO-approved facility. Transgenic arthropods should be clearly identified, securely transported, and stored separately from other arthropods to avoid unauthorized or accidental mixing.
- Contingency plans for destruction of transgenic arthropods in the event of accidental release from, or during transit to, the confined field release site. The plan should include site marking, detection survey, and immediate notification of regulatory authorities.

2.2 Analysis Criteria

The information required in Section 2.1, and appropriate methods as described in the relevant international standards, provide the basis for an analysis of the phytosanitary risk associated with the transgenic arthropod itself that may be presented by the proposed confined field release. Some information may not be applicable depending on the arthropod species and the nature of the introduced transgenic trait and, in such cases, may be waived. The submitted information should be reviewed on a case-by-case basis for its completeness and acceptability. When applicable, this may be done by an expert advisory group.

Authorizations by the NPPO to allow confined field release of transgenic arthropods into the environment should generally be granted only when a determination can be made that the proposed confined field release does not pose a significant phytosanitary risk. In order to make such a determination, the following criteria for the analysis should be met:

- Any transformation vectors associated with the transgenic arthropod that are able to transfer genes and/or cause a risk of disease, damage or injury to plants have been minimized or eliminated from the transgenic arthropod.
- Appropriate confinement measures and detection survey are in place at the confined field release site to adequately protect against dissemination of the transgenic arthropod and/or unmodified arthropods that are released as part of the experimental design.
- Any new property of the transgenic arthropod has a minimal likelihood of a detrimental effect on plant health, other arthropods, and non-target organisms, including consideration of the effects on threatened and endangered species, or on the habitat that could have indirect effects on plant health. Any such effect is limited by the size of the confined field release, appropriate selection of the site, and appropriate reproductive isolation and confinement measures.

- A contingency plan for stopping the confined field release that includes clear lines of authority and requisite actions.

2.3 Authorizations

Authorization for confined field releases should generally be granted when the analysis criteria in Section 2.2 allow for a determination that the proposed confined field release is not considered to pose a significant phytosanitary risk.

- Authorizations to conduct confined field release should be valid for a fixed period of time.
- Authorizations to conduct confined field release should be conditional on the employment of applicable risk management options listed in Section 2.1.4.

2.3.1 Inspection

Authorized confined field release should be subject to inspection by regulatory authorities during the period of the release to verify that the confined field release is carried out in accordance with the authorization conditions.

2.3.2 Information

The applicant should be responsible for reporting any information to regulatory authorities relating to significant changes in phytosanitary risk. Regulatory officials should consider, and where appropriate, ensure corrective action is taken.

2.3.3 Record keeping

Records of all activities related to confined field release compliance, including experimental data and monitoring during the confined field release, should be maintained by the applicant. Records should be made available to the regulatory authority upon request. A final report should be produced and be made available to regulatory authorities upon request.