

Guidelines for Introducing Beneficial Insect-parasitic Nematodes into the United States

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Abstract: Guidelines are suggested to implement the introduction of beneficial insect-parasitic nematodes into the United States from abroad. These suggestions result from experiences and research with these and other biological control agents and from the current need for procedures to import nematodes. Subjects considered are need to import, foreign exploration, taxonomy, shipment, quarantine facilities, permits, host range tests, release, and documentation. Nematodes covered under these suggested guidelines include entomopathogenic species of mermithids, sphaerulariids, aphelenchids, steinernematids, and heterorhabditids. Host specificity and safety tests are discussed. Concern over the possible concomitant introduction of plant-parasitic nematodes, insects, or other pests is expressed. Current information on the treatment of insect-parasitic nematodes by the Environmental Protection Agency and USDA's Animal and Plant Health Inspection Service is presented. These suggested guidelines are presented to stimulate the development of workable protocols for safe introduction of beneficial insect-parasitic nematodes into the United States from abroad.

Key words: biological control, entomogenous nematode, guideline, insect parasite, introduction, nematode introduction, permit form, procedure.

Increased emphasis on biological control of pests in general has resulted in an increase in the use of nematodes as biological control agents. In 1987 there were 88 specific Current Research Information System (CRIS) research projects within USDA's Agricultural Research Service (ARS) and state experiment station systems that included use of nematodes for pest insect control. The increased interest in nematodes for biological control has led to the publication of textbooks on this subject (5,16,20). Because many pests targeted for biological control are of exotic origin, the biotic agents, including nematodes, identified for introduction against these pests often are of foreign origin also.

Inherent in any program involving the use of exotic organisms is the danger of introducing an undesirable species. This hazard has long been recognized in association with programs involving exotic arthropod enemies of weeds and insect pests. As a result, various procedures have been developed as guidelines for the introduc-

tion of beneficial arthropods (1,4,6,12). Guidelines need to be developed for the introduction of insect-parasitic nematodes as biological control agents of insects. In 1978 ARS scientists began developing guidelines for introducing beneficial nematodes, but, because of rapidly changing legal and regulatory actions in the area of introduction of exotic (and genetically engineered) organisms, those guidelines remain unfinished (4). Some of the provisions expressed in the unpublished guidelines are incorporated into this paper.

Existing guidelines for the introduction of plant-feeding arthropods (4,12) should be consulted for procedures for introducing plant-parasitic nematodes for biological control of weeds. Presented here are suggested guidelines for the movement of nematodes into and within the United States as part of the expanding program for the biological control of arthropod pests. These suggested guidelines are designed to insure that every reasonable precaution will be taken to contain and prevent the escape or release of exotic nematodes that may be injurious to agricultural, horticultural, or forestry commodities, to man and domestic animals, or to beneficial arthropods, or in any other way be detrimental to the North American environment. The guidelines are intended

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to apply to all species of insect-parasitic mermithids, sphaerulariids, aphelenchids, steinernematids, heterorhabditids, and other groups that are to be studied or used for the biological suppression of arthropod pests. We hope the suggested guidelines that follow will stimulate discussions among appropriate specialists that will result in more formalized protocols as previously defined (4).

PRELIMINARY STEPS

Determining the need for introduction

Because of the intensive research associated with the introduction of a nematode, an initial evaluation must be made to determine if there is a need to introduce a nematode. If the target pest is already sufficiently suppressed by beneficial agents, the introduction of a nematode parasite may not be worth the research effort and expense required.

Selecting a target arthropod

Unlike many beneficial insects and mites, nematodes have a restricted environment in which they can develop effectively. Consequently, selecting an arthropod as the subject of a nematode introduction project must include consideration of the suitability of the environment of the arthropod for the survival and development of the nematode. Nematodes as parasites have the most potential for controlling soil-dwelling and water-dwelling insects (15,16). Pest species inhabiting arid environments are less likely to have an extended fauna of associated nematodes than are those arthropods inhabiting moist environments during all or part of their life cycle. Furthermore, the impact of the nematode on the arthropod should be before or during the pest's developmental stage that is either the harmful or the reproductive stage. Nematodes that attack a pest after it has reproduced or caused damage are generally not effective control agents.

Foreign studies

When insect-parasitic nematodes are discovered during the course of foreign ex-

plorations, or are otherwise known to attack the target pest in a foreign location, samples of the potential host material collected should be examined, before shipment of the material, to determine whether nematodes are present and the percentage of parasitism. Other potential hosts in the collection area (particularly beneficial arthropods) should be examined to determine whether any of these may be attacked by the same nematode. Specimens of nematodes obtained in foreign studies, along with specimens of their hosts, should be retained as vouchers for further study as may be needed.

Taxonomic considerations

Before shipping living material from overseas, a preliminary identification of the nematode should be made, if possible, to determine the group or family to which the nematode belongs. Such identification *must* be made in quarantine or other receiving facility before further shipment or release. Identification will affect subsequent research and introduction procedures and documentation requirements, including the issuance of shipping permits for export (for some countries), importation, and interstate movement.

SHIPMENT PROCEDURES

Insect-parasitic nematodes are obligate arthropod parasites that are specialized to the point where they cannot feed on plants. Proper identification of exotic insect-parasitic nematodes will insure that no plant-parasitic nematodes are included inadvertently in shipments.

For guideline purposes, insect-parasitic nematodes generally can be treated in four groups: 1) Mermithidae. The highly specialized insect-parasitic nematodes of this family may be considered for importation after identification to family. Adults, post-parasitic juveniles, or eggs separated from the host insects may be placed in sterilized soil or sand in an approved container for shipment under conditions stated in the permit, or they may be shipped within parasitized hosts, again under conditions stat-

ed in the permit. If live insects are included, entry permits usually require initial receipt in an insect quarantine facility. 2) Sphaerulariidae. Infective stages of these parasites separated from the host insect and placed in sterile soil or sand may be sent to approved facilities for further identification and study. As with mermithids, if live infested host insects are necessary to insure survival of this type of nematode, the shipment must be made under conditions stated in the permit, usually to include initial receipt in an approved insect quarantine facility where the nematodes can be retrieved from the hosts for further shipment. 3) Steinernematidae and Heterorhabditidae. Dauer, or resting stage, juveniles of these nematodes can be shipped in sterile sand or soil or on artificial media (5) or in the insect host and under conditions stated on the permit. Host range studies, discussed in the section on safety considerations, should be conducted under strictly controlled conditions before release. 4) Aphelenchoidea and other groups of insect-parasitic nematodes. Nematodes of groups whose biologies are generally less well known than those of the other groups noted may be treated in the same manner as sphaerulariid nematodes. If not already known for the family being imported, the presence or absence of microbial associates in nematodes of lesser known groups should be determined before their shipment from domestic containment facilities.

With these general safety considerations in mind, procedures for shipment of exotic materials are summarized as follows:

Permits

Before any shipments of nematodes or infested host material can be made, appropriate permits must be obtained.

Export permits: Some countries require export shipping permits for all items including living organisms. Whether this requirement is needed should be determined during the early stages of the project.

Importation permits: Appropriate shipment (importation) permits must be obtained from Plant Protection and Quar-

antine (PPQ) or Veterinary Services (VS) of USDA's Animal and Plant Health Inspection Service (APHIS) offices for shipment of living material into the United States.

For nematodes attacking plant pests, a PPQ Form 526 serves as both permit application and final permit for importation (14). The permit application form should first be sent for the approval of the regulatory officials in the state(s) in which importation is planned. After approval, the application is sent by the state officials for final approval by PPQ and PPQ then issues permit labels for the shipment(s) as may be required. Standard safeguards of the permit are noted on the reverse of the PPQ Form 526, and additional conditions may be stipulated by state or federal offices as part of the permit.

Nematodes associated with dung or that attack vectors of animal diseases require a VS Form 16-3 for VS approval for importation.

Copies of these forms may be obtained from APHIS, PPQ or VS offices, Federal Building, 6505 Belcrest Road, Hyattsville, Maryland 20782.

Interstate shipment permits: If further shipment of the imported nematodes from the initial receiving facility is planned, specific permits for interstate shipments must be obtained from state and APHIS officials, and permit labels should be made available for use by the facility where the organisms have been cleared for release from containment.

Quarantine facility notification

In many cases, shipments can be made directly to the permittee's facility or the PPQ facility on the supplied permit label. A copy of the permit is generally sent to the designated port of entry. If receipt in a quarantine facility is advisable or otherwise required by the permit, the appropriate quarantine receiving station should be contacted during the initial planning stage to make arrangements for the receipt, clearance studies, and eventual re-shipment of the exotic nematode material

to the final recipients. Shipments of live material generally should not be sent to a quarantine facility without first obtaining an agreement to at least a tentative schedule of shipments and a commitment by the receiving station for proper handling of the nematode material.

Shipping containers

Specimens must be placed in a sturdy container within a second carton with adequate packing and, usually, a refrigerant to keep the contents cool. Various methods for packaging and shipping beneficial insects have been described (2). These techniques can be used for host insects and can be adapted readily for other living organisms.

Contents

Before packing nematodes or nematode-infested hosts for shipment, all unsterilized soil (if possible), plant material, unnecessary arthropods, and all extraneous material must be removed. Only material essential for the survival of the host or nematode should be included in any shipment of foreign origin. If it is necessary to ship nematodes in soil to obtain viable specimens, the soil must be autoclaved or otherwise sterilized before use. Any shipment including soil of foreign origin requires special additional permits from APHIS-PPQ. Any inadvertently included plant or insect material and soil should be autoclaved by the recipient as soon as possible after receipt of the shipment.

Interstate shipment

Domestic shipments of nematodes should also be devoid of all extraneous material. Only essential material should be included in the container. Interstate permit requirements noted earlier in this shipment procedures section are discussed in the release section.

SAFETY CONSIDERATIONS

Identification of the nematode

An authoritative identification of the nematode being introduced is essential to

determine the relative safety of the organism. Identification is necessary to determine 1) the existing published information relating to the nematode and related species and higher taxa, 2) biological information pertaining to the nematode, 3) its relationship with other nematodes, 4) its known hosts and host range, and 5) the subsequent direction of the research to determine the safety of the organism.

Host range of nematode

In general, the biotic agent introduced should be as host specific as possible to reduce the risk that the exotic agent will attack desired or beneficial organisms. Some knowledge must be obtained of the actual or potential host range of any exotic nematode species or strain being considered for introduction and release. Host range data may be obtained from the literature, from field surveys, and from laboratory tests. Careful consideration must be made of the environmental effects of introducing an exotic nematode with a reportedly wide host range.

Containment research

Host acceptance studies: It must be established early in the project that the exotic nematode will infect the target domestic pest arthropod(s). Because of quarantine regulations of other countries, the domestic pest arthropod usually cannot be sent to a foreign location for initial host acceptance tests. Consequently, acceptance tests may have to be conducted at a domestic quarantine facility. Therefore, when the nematode has been cleared for entry into the United States, it is essential to demonstrate first that the nematode will accept the domestic target pest as a host.

Cultures required for identification and description of the nematode: Since living specimens of one or more developmental stages may be necessary for identifying or describing the species, a culture of the nematode may be required. If the culture can be maintained on artificial media, normal precautions to prevent escape of viable nematodes are required. If the nematode

must be cultured in the arthropod host, the culture must be maintained under strictly confined conditions to prevent the escape of the nematode in parasitized hosts. This is especially true if the arthropods required are of foreign origin and facultative hosts are not available. In these cases, the treatment of the arthropod hosts is covered by established regulations and the cultures must be maintained in an approved insect quarantine facility.

Host specificity and safety studies: Release of an exotic nematode poses the risk of infecting nontarget organisms. Host specificity and other safety tests must be conducted under controlled conditions to determine the host range of the nematode and to identify potential dangers that may arise from introducing the nematode under consideration. These studies should be conducted in indoor facilities. Any necessary tests under outdoor conditions must be done under stringently controlled conditions approved by state and federal regulatory officials; an example of such conditions has been described (18). For host specificity studies, a list of test arthropods, including beneficial species, should be developed on a case-by-case basis, generally to include representatives of certain specified arthropod families, depending upon the nematode involved. In some instances, invertebrates other than arthropods may be included in these tests. When possible, beneficial and other organisms associated with the host of the nematode in its foreign habitat should be examined during the foreign exploration phase to determine whether other hosts of the nematode exist within the same endemic area.

Recently, research directed toward the possible introduction of insect-parasitic nematodes from foreign countries has shown that beneficial insects can be affected (8-11,13,17,18). In other research involving animal safety tests, no evidence of infectivity, pathogenicity, or toxicity in any of the test animals has been documented (7,19,21). Studies such as these may be needed when contemplating the introduc-

tion of new, exotic nematodes into the United States.

Research on bacterial associates: In July 1987 the Environmental Protection Agency determined that all strains and species of insect-parasitic nematodes of the genera *Steinernema* and *Heterorhabditis* with their associated bacteria *Xenorhabdus nematophilus* (Thomas and Poinar) and *X. luminescens* (Thomas and Poinar) are exempted from registration requirements under the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA). These bacteria are strongly host dependent and are not known to survive for long periods outside their nematode symbionts or secondary host insects; it is unlikely they could or would cause infection in mammals. Therefore, tests on mammals would not be necessary unless the nematode to be released is found to be associated with a bacterium other than a species of the genus *Xenorhabdus*.

Because mermithid nematode parasites of insects do not serve as vectors for bacteria, they were also exempted from registration requirements under FIFRA in 1976. These and other nematodes are classified as macroparasites, and their movement is regulated by the USDA.

Basic biology studies: If the biology of the newly imported nematode is unknown, biological studies are highly advisable. They should be conducted under controlled conditions or in quarantine facilities if such are available for extended use. Such research would develop information applicable to the release, distribution, recovery, and evaluation of the nematode if it is cleared for release. Even if not cleared, the information obtained would still be valuable for future projects with closely related nematodes.

RELEASE

Working group

It is recommended that a committee or working group be established, similar to the former Working Group on Biological Control of Weeds (12), which is now the interagency Technical Advisory Group for

Introduction of Biological Control Agents of Weeds under the auspices of USDA's APHIS-PPQ. The proposed committee, possibly designated as the Working Group for the Introduction of Beneficial Nematodes, would consist of specialists representing several disciplines that are directly or indirectly involved with nematodes, quarantine, agriculture, and the environment. This working group would be responsible for assisting the researcher to establish case-by-case lists of organisms to be included in safety and screening tests to demonstrate the specificity of the exotic nematode species being considered for field release. Also, the working group would review the research data developed for the nematode and recommend either approval or disapproval of the introduction of the nematode or require additional research before a final decision could be made.

Permits

After receiving approval for the field release of the exotic nematode, either from the proposed working group or, in its absence, from research administrators of the research facility involved, state permission and APHIS permits must again be obtained. For nematodes attacking plant pests, a PPQ Form 526 serves as both an application form and final permit for interstate shipment or field release of the organism (14). Approval of the regulatory officials in the state(s) in which releases are planned are required before final approval by PPQ. Nematodes associated with dung or that attack vectors of animal diseases require a VS Form 16-3 for VS approval for shipment and release. Procedures for obtaining permits were discussed in the shipment procedures section.

DOCUMENTATION

The search for exotic organisms, and their importation and release, provides an excellent opportunity to collect and store information of value to current and future biological control practitioners and to taxonomists and ecologists. For these reasons,

and for quarantine and regulatory purposes, adequate recording of the movement, release, and recovery of biotic agents and the retention of specimens to serve as vouchers are essential.

Documentation of shipments and releases

The USDA has developed forms for documenting the importation (AD-941), release from quarantine (AD-942), and subsequent nonquarantine movement (AD-943) of introduced biological control organisms (3). The forms were designed to provide source information for recipients of exotic organisms and feedback information for the shipper or supplier, as well as records for inclusion in USDA's computerized database on "Releases of Beneficial Organisms in the United States and Territories (ROBO)." Supplies of the forms may be obtained from the ARS Biological Control Documentation Center, BARC-E, Beltsville, Maryland 20705. Use of these forms, or a similarly detailed recording system, will reserve information relating to collection, movement, and release of exotic nematodes that is essential for effective follow-up evaluation studies.

Voucher specimens

Shippers and recipients of exotic organisms should retain properly prepared specimens representing shipped or released nematodes to serve as voucher specimens documenting importation or release. These specimens are essential for later taxonomic study if identity of the released nematode is ever questioned or if it appears possible, following major taxonomic revisions of the group to which the nematode in question belongs, that several cryptic species may have been released. Nematodes should be preserved in a solution of 3% formaldehyde, 2% glycerine. If sufficient specimens exist representing a nematode newly introduced into the United States, some should be sent for deposit as vouchers in the USDA Nematode Collection, BARC-E, Beltsville, Maryland 20705. Specimens of the original exotic host of the nematode should also

be retained by the researcher for possible later verification of identity. Preparation techniques for arthropod specimens have been described (22).

Publications

Documentation also includes publication of research and other results of the project. The early publication of a taxonomic description of a new or undescribed exotic species of nematode is desirable. Studies of the biology and host specificity of the species or strain and data on its introduction and location of voucher specimens also should be published.

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