PREDICTING HOST RANGE OF BIOLOGICAL CONTROL AGENT CANDIDATES FOR INTRODUCTION

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Biological control, the use of living organisms to control pests, has proven to be an important tool of integrated pest management, an alternative to chemical pesticides in many cases. Historically biological control has been considered an environmentally safe approach to pest control; however, in the last 20 years, increasing concern about the introduction of all kinds of exotic organisms and their potential effects on non-target organisms in the new environment has arisen, and biological control has received increased scrutiny. Regulations for introduction of natural enemies into new countries and the risk assessment procedures vary from country to country. International, regional, and national initiatives have developed standards to ensure the safety of biological control projects. An example is the United Nations Food and Agriculture Organization (FAO) Code of Conduct for the Import and Release of Exotic Biological Control Agents, which was signed by FAO member countries in 1995 (FAO 1996). Several countries have developed new legislation or revised existing regulations on the introduction of new organisms to harmonize with the FAO code of conduct. Currently the majority of countries require evidence that any biological control agent released, especially biocontrol agents for weed control, will not have adverse economic and or environmental impacts in the country of introduction. If there is a danger of attack on non–target species, then a risk / benefit analysis must be done. Host specificity of biological control organisms has been the primary and often the only criteria that scientists and regulators use for assessing likelihood of non-target impacts of biological control introductions. Host specificity tests typically measure the potential of the control organism to complete its life cycle on the target organism and on the non–target organism that it attacks (eats, parasitizes, or infects). However, it may be limited as a safety criteria because a control organism may harm a non–target organism in several ways: a direct trophic interaction, when the control organisms consumes a non-target organism; a direct interference in competition, and an indirect interaction, when the control organism and the non-target organism interact via intermediate species, such as a shared host. Here we will give an overview of the present risk assessment procedures for different kinds of natural enemies, i.e., phytophagous, entomophagous, and entomopathogens arthropods, and then we will present a literature-based protocol to predict host ranges of parasitoids which are candidates for introduction.

Phytophagous Arthropods

Pre-release tests to determine the susceptibility of non-target plants to control agents have been part of biological control of weeds for over 60 years. Determining the host range of a biological control agent shows whether the candidate for introduction has the ability to feed, oviposit and /or develop on plants other than the target weed. At first, the standard method was to perform no-choice tests with plants of economic value. This kind of test evaluated whether a particular plant tested was at risk, but did not provide information about the host range of the biocontrol agent. Since 1970, tests have been set up to determine the boundaries of the host range, and to predict whether plants with economic or aesthetic value are inside that range. This method, called centrifugal phylogenetic evaluation, starts with plant species closely related to the target weed and continues with ones more distantly related until the host range has been delimited phylogenetically. This has become the standard method for determining the host range of arthropod biocontrol agent of weeds. Based on the existing literature there is little or no evidence that this approach has ever failed, which suggests that the prediction method is good. However, complete specificity is relatively uncommon and some non-target plant species may be subject to some minor feeding and/or oviposition by the agent, but the damage is insignificant or the larvae fail to develop completely and the agent is allowed to be released. When the non–target species damaged by the candidate is a weed also, the impact may not be considered critical. However, if the non-target at risk is considered a desirable species, then determination whether the agent is safe to be release in the field is fundamental, and further tests should be conducted to determine if the agent could persist on the non-target species. These follow up tests have been called continuation trials and consist of returning adults that have completed development on one plant species during choice or no choice tests, to a fresh plant of the same species in no-choice trials, to test whether the non–target species can support successive generations of the agent. A plant species that can support successive generations of an agent is potentially an alternative host, and therefore at risk. The most difficult result to interpret is when adults that have developed on a non–target species are able to oviposit and the larvae can complete development on non-target species, but the population declines over subsequent generations. It is important to consider that in the field conditions the potential total damage to a plant is a sum of the damage caused by all generations, including insects that migrate from the target weed and so even a small damage over many generations might be significant.

Entomopathogen Arthropods

Microorganisms

For pathogens used for biological control introduction programs, the majority of examples are for weed control. Approaches similar to those for phytophagous arthropods are used to evaluate host range and potential risk to non-targets. For microorganisms that attack insects, or even other microorganisms (antagonists), used as biological control agents, protocols for evaluating risk to non-target organisms are not established. There has always been concern about the side effects of entomopathogens on commercially important organisms, such as bees, silkworms, earthworms, and several methodologies for testing these species are available. However, with increasing interest in use of microorganisms in biological control introduction programs for arthropod or plant pathogen pests, concern about non-target effects on all kind of native species, and especially on those important for pest control (other biological control agents) has increased. The potential risks associated with the use of microorganisms as a biocontrol agents can be group in four classes: 1) displacement of non-target species, 2) allergenicity to humans and other animals, 3) toxigenicity to non-target organisms, and 4) pathogenicity to non-target organisms. These unintended non–target effects are independent of whether the pathogen agent is indigenous or non –indigenous, naturally occurring or genetically modified. The tests used to predict risks are able to detect only infectivity/toxigenicity to non-targets, because displacement and other side effects are difficult to assess For entomopathogens, various approaches have been used to evaluate host range, and recently, guidelines for evaluating effects of entomopathogens on non–target species were published. Some of the important points will be described below.

The range of species that an entomopathogen is able to infect, usually under optimical conditions and often called physiological host range, is determined in the laboratory and shows which species the pathogen can infect when encountered. Typical effects measured include a combination of mortality, infection, and/or pathogen reproduction. Ecological host range is the range of hosts infected by the pathogen under field conditions, and it is of primary importance for assessing impact on non-targets. The effects of entomopathogens on non-target species are not only affected by infectivity, but also can be influenced by the ecological niches occupied by the pathogen and potential hosts. Knowledge of physiological host range can help delineate the potential hosts in the ecological host range. However, it is very common that hosts that are infected in the lab are never found infected in the field, because the complex biotic and abiotic interactions that occur in the field are difficult to simulate in the laboratory. Thus extrapolation of laboratory data to the field must be done with caution. Careful design of laboratory, semi-field and field studies can provide useful information for risk assessment. When carrying out tests to evaluate host range of microorganisms, both positive (inoculated, susceptible hosts) and negative (non–inoculated, susceptible hosts) controls must be included. In addition, for all kind of tests, it is important to have methods for rapid and definitive identification, diagnosis of disease, isolation of the pathogen, and quantification of pathogen impact. Other parameters besides the mortality should be evaluated, including the ability of the pathogen to replicate within the non–target host, the possibility of latent or sub-lethal infections causing reductions in longevity and fecundity. Non-target infections may be abnormal if the species in question is a marginal host. The non-target species tested should be ones potentially present in the field rather than ones readily available from lab colonies. However, working with wild populations is often not easy because of problems with collection and rearing in the lab. For biological control introductions, pathogens are often released in small quantities and are expected to propagate in the environment within the host population. Thus studies should focus on determining the potential of the pathogen to establish and to persist by infecting non-target populations. Emphasis should be placed on those organisms that live in the same habitat as the target pest. Useful information can be obtained by studying the epizootiology of the pathogen in its area of origin and evaluating which hosts are infected in the field, how well the pathogen persists, and what non-target species are normally exposed to the pathogen. These data would indicate the types of hosts likely to be infected in the field, together with information on taxonomy and ecology of insects infected in the field. Despite the value of these studies, they are costly, take time, and are difficult to perform because levels of both host and pathogen often are very low in the area of origin. As an alternative, data on the ecological specificity of closely related pathogens could be used to gain some idea about the potential host range in the field.

Entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* (Nematoda: Rhabditida) and their symbiotic bacteria have emerged as excellent insect biological control agents and there is an increasing interest in the discovery of new species/strains and in the use of EPNs for pest control. Although EPNs have been used primarily in inundative approaches, they have been used in biological introductionS, e.g., for the control of mole crickets in Florida, USA. These nematodes possess special features which dististinguish them from other organisms used in the biological control of pests. Because of the unique dual character of the nematode-bacterium complex, different regulatory approaches have been adopted in different countries. They are considered microorganisms in some countries and macroorganisms in others, and therefore are regulated differently. Currently there is no harmonized regulation for the introduction, release, and commercialization of entomopathogenic nematodes.
These nematodes attack a wide range of insects in the laboratory where host contact is assured, environmental conditions are optimal, and ecological or behavioral

barriers to infection are lacking. Some species infect more than 250 species of insects from over 75 families in 11 orders. However, under field conditions, the nematodes tend to be quickly inactivated by environmental extremes (e.g., desiccation, UV radiation, temperature) and behavioral barriers restrict nematode efficacy to a few selected hosts or host groups. Negative impacts are often limited to treated fields because of the low mobility of entomopathogenic nematodes, the cryptic environments in which they live (soils, other plant growth media or tunnels inside plant material), and low survival on foliage. Although several short-term laboratory and field studies have documented safety and or minimal adverse effects of entomopathogenic nematodes to mobile, above-ground, non-target invertebrates, their effects on soil microfauna and flora are largely unknown. Some recent studies suggest that entomopathogenic nematodes could have significant impact on soil food webs and their ecological functions. Because long-term studies on the impact of nematodes on soil invertebrates are lacking, it is premature to conclude that these nematodes are safe for all the non-target invertebrates in all environments.

Entomophagous Arthropods

Historically arthropod parasitoids and predators have not been subject to host range testing. However, in the last few years because of the current concern about the impact of exotic species on native species these biocontrol agents have also received increased scrutiny. Formal host range evaluation has been required for introductions of arthropod parasitoid and predators in several countries, such as Australia, New Zealand, South Africa, and United States. There have been discussions about whether host range of parasitoid and predators should be evaluated with the same approach as used for weed control agents. However, collecting, rearing and testing non-target arthropods are often much more difficult than doing this for plants, which make tests for arthropods particularly expensive in time and labor. Such difficulties may put experiments in quarantine or in the field in the source region beyond the budgets of many projects, particularly in less-developed countries. To overcome these problems, we propose a protocol using the literature as the main source of information to assess host range of parasitoids, which are candidates for introduction. Directly measuring the host range of a given parasitoid species is rather difficult, and publications on host ranges of parasitoid species are limited. On the other hand, it is relatively easy to determine which parasitoids attack a given host species by collecting and rearing various host stages, and the literature contains many studies of this sort. We propose that data on parasitism should be obtained in the area of origin from potential hosts phylogenetically close to the target pest and more distantly related species, which resemble the target pest in behavior or ecology (e.g., feeding niche, habitat preference, phenology). The idea is to use phylogenetic and ecological similarity to predict host range in the region of introduction. Source regions often have pest species phylogenetically and ecologically close to the target pest. Because they are pests, their parasites have been often been surveyed. Data from such surveys can be used to test whether the candidate attacked non-target species.

Because sampling effort varies among surveys, some assessment of effort is needed. Number and geographical coverage of sites, survey duration, and the number of the relevant host stage collected, should be taken into account, from the last variable, one can calculate a crude estimate of the confidence that a potential host was not indeed attacked by the candidate. If no parasitized hosts were detected, one can say with 95% confidence that parasitism by the candidate would have to have been below $1-0.95^{(1/n)}$, where n is the number of host individuals examined. In fact, we use negative evidence concerning attack by the candidate, whose host range one wants to evaluate. By compiling studies for a variety of host species, one can at least delineate these taxa unlikely to be attacked by the candidate in the new environment and only when necessary quarantine tests should be performed. Using a retrospective case study of a parasitoid introduced into North America, we showed that, at least in this case study, the predicted host range for North America matched the actual host range found in the field. This suggests that a careful literature review could be used as the main source of data on host range of parasitoid species proposed for introduction into a new environment. This model may not be used to evaluate host range of predator arthropods used as biological control, because it is very difficult to identify the prey they eat under field conditions and consequently, data from the literature about predator host range is difficult to find.

What risks to non-target species should be acceptable?

Finally, we would like to address what constitutes acceptable risk to non-target organism. Acceptability of a risk to non-target species is a societal decision that can vary from country to country and may evolve with changing societal values and scientific knowledge. However currently with some international agreements such as the Biodiversity Convention and its Agenda 21, which states that no introduction of exotic species should be allowed if any native species will be threatened, the power to accept risk is more limited. To determine what risk is acceptable requires cost-benefit analyses that should include ecological, ethical , and economic issues. The decisions themselves are not scientific; however, science can provide information to help to make the decisions.