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Commentary

A review of introductions of pathogens and nematodes for classical biological control of insects and mites

Ann E. Hajek [a,*], Michael L. McManus [b], Italo Delalibera Júnior [c]

[a] *Department of Entomology, Cornell University, Ithaca, NY 14853-2601, USA*
[b] *USDA Forest Service, Northeastern Research Station, Hamden, CT 06514, USA*
[c] *Department of Entomology, Plant Pathology, and Agricultural Zoology, ESALQ-University of São Paulo, Piracicaba 13418-900, Brazil*

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Abstract

Compared with parasitoids and predators, classical biological control programs targeting arthropod pests have used pathogens and nematodes very little. However, some pathogens and nematodes that have been introduced have become established and provided excellent control and have been introduced in increasing numbers of areas over decades, often after distributions of pests have increased. We summarize 131 introductions, the majority of which have occurred since 1950. The most commonly introduced microorganisms have been fungi, viruses and nematodes, although microsporidia, bacteria and oomycetes have also been introduced; among these groups, viruses were the most successful in establishing followed by nematodes, fungi and microsporidia. All major orders of insects and prostigmatid mites have been targeted and in 63.6% of the programs the pests being targeted were invasive species and not native. Pathogens and nematodes yielded excellent success in establishment against sawflies and wood wasps (100% of programs) and 40–48% establishment among other host orders. Classical biological control has been used for long-term control of arthropod pests on islands almost as much as in mainland areas. It has been used most frequently in perennial systems and highest rates of establishment of arthropod pathogens and insect parasitic nematodes were documented from forests (63.0%) and tree crops (66.7%). One explanation for the low number of releases of arthropod pathogens and insect parasitic nematodes has been confusing and difficult regulations but recent changes and institution of the FAO's Code of Conduct is expected to improve scientists' ability to introduce microbial natural enemies for classical biological control.
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1. Scope and background of the review

Pests are constantly being introduced to new areas either naturally or accidentally, or, in some cases, organisms that are intentionally introduced become pests. The exponential growth of global trade and increase in efficiency and availability of human travel have increased the numbers of exotic pests that become established in new areas every year; this has become a critically important ecological problem around the world. For example, exotic species comprise about 30–40% of the insect and mite pests in the United States (Pimentel et al., 2000) and the activity of these exotics that become pests results in huge monetary losses around the world (Pimentel, 2002). In addition, exotic species that become established in new areas can seriously alter native communities. In the United States alone, 42% of the decline in native species that are threatened or endangered is thought to have been due to competition with exotic species (Groves, 1986). Because of these negative impacts, exotic species are often called invasive or alien.

One approach that has frequently been used to reduce the impact caused by invasive arthropod pests is classical biological control. Classical biological control is defined as "The intentional introduction of an exotic biological

* Corresponding author. Fax: +1 607 255 0939.
E-mail address: aeh4@cornell.edu (A.E. Hajek).

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control agent for permanent establishment and long-term pest control" (Eilenberg et al., 2001). Classical biological control is also sometimes referred to as 'importation of natural enemies' or 'introduction/establishment' and is especially appropriate when an alien species is introduced into a new area, where it becomes an established pest. Classical biological control is often based on the premise that in the new area, the introduced species has escaped from the natural enemies that regulate its populations in its area of endemism (= the 'enemy release hypothesis') and that the invasive pest will be naturally controlled once reunited with its natural enemies (Keane and Crawley, 2002). However, our review shows that classical biological control programs have also used exotic pathogens and nematodes to control indigenous pests and some of these programs have yielded successful establishment of the natural enemies.

The first program of classical biological control that brought widespread attention to the great potential success possible with this method targeted the cottony cushion scale, *Icerya purchasi* Maskell. This pest was introduced to California around 1868 and, by 1886, the new and growing California citrus industry was being decimated by damage caused by this scale (DeBach, 1974). The cottony cushion scale was successfully controlled by the vedalia beetle, *Rodolia cardinalis* (Mulsant), a coccinellid introduced from Australia in 1889 (Caltagirone and Doutt, 1989). Since then, this control strategy has been used extensively to introduce arthropod predators and parasitoids to control arthropod pests and phytophagous insects and plant pathogens to control weeds (Clausen, 1978; Greathead and Greathead, 1992; Julien and Griffiths, 1998). As long-term solutions against invasive insect and mite pests (i.e., use in classical biological control programs), arthropod-pathogenic microbes and nematodes have been used much less frequently than introductions of parasitoids and predators (Hajek, 2004; Hajek et al., 2000; Fuxa, 1987). However, some pathogens and nematodes that have been introduced for classical biological control have become established and have been very successful in providing substantial and long-term control of pests (Goettel and Hajek, 2001).

Researchers have been trying to determine under which circumstances classical biological control introductions of predators and parasitoids for control of pestiferous arthropods (e.g., Stiling, 1993) or phytophagous insects to control weeds (e.g., Sheppard, 1992) work best and for what reasons these agents sometimes fail to become established. Such quantitative analyses have never been conducted for programs introducing pathogens and nematodes. Fuxa et al. (1998) described characteristics of pathogens well-suited to classical biological control and other control strategies and defined criteria for choosing entomopathogens to use for classical biological control. Guidelines for using exotic pathogens as classical biological control agents were presented by Hajek et al. (2000), including methods for documenting establishment and impact. However, a comprehensive review of the historical releases of pathogens and nematodes as classical biological control agents and the performance of past programs has been lacking. Such a review is necessary for assessing the conditions that have been most successful in establishing exotic pathogens and nematodes for classical biological control.

References with abstracts on early attempts of biological control of insects with entomopathogenic fungi were compiled by Baird (1958), including some programs introducing exotic species of arthropod pathogenic fungi. Burges and Hussey (1971) and Burges (1981) listed 41 examples, where pathogens were introduced into insect populations but many of these examples were not intended for classical biological control, but rather were releases intended only for short-term control (i.e., use as biopesticides). As compared to importations of predators and parasitoids, many references to pathogen and nematode introductions do not report whether the pathogen was confirmed as absent prior to their introduction. Another problem is that the species names and taxonomy of many of the pathogen and nematode species involved in these early publications have been changed or identities of species involved are in question. For the classical biological control strategy, microbes must be considered strain by strain, since some species are distributed worldwide and strains of the same species from different geographical areas can vary in terms of virulence and host range. Pathogen strains often have not been characterized and cannot be separated using morphology or developmental patterns. Thus, identifying different strains of microbes instead of species is challenging and usually requires use of molecular methods. If different strains of the species being introduced are native to an area, it is important that methods allowing discrimination among strains are used to evaluate establishment.

We recently published a catalogue that summarizes documented introductions of pathogens and nematodes for classical biological control of insects and mites (Hajek et al., 2005). This catalogue includes data on pest and pathogen origin, years of liberation, and a summary of establishment and success. In this review, we have synthesized the information assembled in the catalogue. The goal of our review is to learn from an overview of these individual programs how best to improve the success of classical biological control using arthropod pathogenic microbes and insect parasitic nematodes. The general pattern of past programs shows what can be achieved when using this pest control approach.

2. Criteria for including and rating programs

All intentional releases of entomopathogens listed in the catalogue were used in the analysis presented here. Since the catalogue was printed, we have learned of other releases and the following additions have been included in this synthesis. The *Oryctes rhinoceros* virus from Kerala, India was successfully introduced against *O. rhinoceros* L. on Androth Island, Lakshadweep, India (Gopal et al., 2001) in 1988. A Serbian isolate of *Zoophthora radicans* (Brefeld)

Batko was released for control of *Empoasca fabae* (Harris) in New York, USA in 1990–1991 but did not become established (Hodge et al., 1995). The bacterial pathogen *Paenibacillus popilliae* (Dutky) from the United States was released for control of *Popillia japonica* Newman on Terceira Island in the Azores in 1990–1991 but did not become established (Mendes et al., 1994). In addition, we realized that by mistake the years for the release of *P. popilliae* in Kiribati were 1995–1996 (Theunis and Teuriara, 1998) and not 1976, as reported in the catalogue. Also, in the catalogue *Heterorhabditis heliothidis* (Khan, Brooks and Hirschmann) is listed but this name is no longer correct and should instead be *Heterorhabditis bacteriophora* Poinar (Kaya and Stock, 1997). Although this species of nematode already occurred in Australia when it was introduced from New Zealand, this program is still included in the catalogue as an example of an introduction of an exotic strain.

Criteria used for including programs in the catalogue and for categorizing programs and evaluating their success are discussed in the catalogue but also described briefly below. In this paper, the term 'program' refers to release of one species of agent in one geographic area. When creating the catalogue, it was often difficult to determine whether a release program fit the classical biological control approach, particularly when a program was implemented many years ago and/or not thoroughly documented. Thus, we used the following criteria for including programs in the catalogue and in this analysis:

1. The target pest was an insect or mite.
2. The species, strain or biotype of the microbial pathogen or nematode that was introduced was exotic (non-native) to the area of introduction.
3. The intent of the program was to establish the pathogen in the release area, hopefully resulting in long-term (and not temporary) control. For inclusion in the catalogue, there had to be some documented evidence that permanent establishment of the pathogen or nematode was a goal of the program and long-term control was either investigated or discussed. We included some older, poorly documented programs even though information was meager when we were able to determine or could infer that the goal of the program was establishment.

We did not include examples of early widespread introductions of entomopathogens that were later shown to be questionably pathogenic, or widespread introductions where contaminants were probably released instead of the intended pathogen. At the beginning of the last century, several programs focused on the redistribution of (1) non-pathogenic microbes (especially fungi) that were probably accidentally introduced along with the insect host, or (2) indigenous strains of microbes observed infecting the introduced pest. Although some authors listed these cases as pathogen introductions, they have not been included in our analysis. Examples of releases of indigenous strains include: attempts to spread three *Entomophthora* species against the spotted alfalfa aphid *Therioaphis maculata* (Buckton) in 12 counties in California, USA (Hall and Dunn, 1957a,b, 1958); artificial dissemination of *Entomophthora* (= *Empusa*) *erupta* (Dustan) Hall to control the green apple bug, *Lygus communis* Knight (Dustan, 1923); investigations to accelerate the spread of *Z. radicans* (= *Entomophthora sphaerosperma*) to control the European apple sucker (*Psylla mali* Schmidb.) within localities in Nova Scotia, Canada (Dustan, 1924; MacLeod, 1963; Baird, 1958); releases of brown tail moth (*Euproctis chrysorrhoea* L.) larvae infected with the fungus *Entomophaga aulicae* (Reichardt in Bail) Humber in Maine and Massachusetts, USA (Speare and Colley, 1912); and the colonization of *Aschersonia aleyrodis* Webber in populations of the citrus whitefly (*Dialeurodes citri* (Ashmead)), the cloudy winged whitefly, (*Singhiella citrifolii* (Morgan)), and other whiteflies in Florida (Berger, 1906; Osborne and Landa, 1992). Examples of some of the introductions of species that were questionably pathogenic are discussed in Carruthers et al. (1996) and Tanada and Kaya (1993).

Summaries of results from introductions are presented in the catalogue and whenever possible, establishment, persistence and control are briefly discussed. However, in the major analyses presented in the paper, each program was rated according to whether the organism released became established, failed to establish, or if the fate of the introduction was unknown. Unfortunately, detailed summaries of results for many programs are not available for a variety of reasons. In some cases, often for earlier programs, results were simply not reported and we cannot tell if data on establishment were recorded after releases. For some recent programs, perhaps adequate time has not transpired since the release to see an effect. For some biological control programs, at least several years after an introduction are necessary before it is considered that the system has stabilized and evaluation of releases will be accurate. In general, we considered that a pathogen or nematode species was established if it was recovered over a time period after release that would have been adequate for reproduction and reinfection to have occurred in the host population (ca. $\geqslant 2$ years), preferably with some indication of long-term reoccurrence of infections. We decided not to attempt to summarize success in achieving control in individual programs because, in the vast majority of cases, historical data were either lacking, too subjective or inadequate to make comparisons among programs.

Often sampling in release areas was not conducted prior to releases to confirm that the pathogen was not already present; in fact, it was rarely reported whether pre-release sampling was conducted or not. It is possible that the number of failed releases has been underestimated because if an introduction fails, we feel that it is often not reported. However, these issues are not specific to classical biological control using pathogens but would also be relevant to releases of parasitoids and predators for control of arthropod pests and phytophagous arthropods for weed control.

We took a conservative approach to summarizing results and, unless otherwise stated, percentage successful establishment has been calculated by dividing the number of established programs by the numbers of programs reporting lack of establishment plus the number of programs not reporting results.

Classical biological control programs are listed separately if they occurred in areas geographically isolated from one another even within the same large country. For example, introductions of the nematode *Romanomermis culicivorax* Ross and Smith from Louisiana to control species of mosquitoes in Maryland and California are listed as separate introduction programs. Introductions to islands that were very isolated were also counted as separate programs.

The type of habitat in which the target pest occurred was also evaluated based on some broad categories that we established. Arthropod pests categorized under herbaceous crops, forest trees, tree crops and rangeland were always feeding on plants above ground. The designation for soil was always for root-feeding arthropods that often were feeding on perennial plants. Our designation of tree crops included all woody ornamentals, coffee, palms, coconuts and other woody perennial crops.

When analyzing data in the catalogue, we counted numbers of programs undertaken as well as numbers of pest species targeted. These numbers are at times very different because in some cases, one species of pathogen or nematode was released in numerous locations, sometimes targeting different or multiple pest species at different locations.

3. General statistics

We identified 131 classical biological control programs which involved the introduction of pathogens or nematodes against 76 insect species or groups of species (in a few cases, a group of species, e.g., lecaniine scales, was listed) and three species of mite pests. Almost half of the microbes released (48.1%) became established in the new habitat and establishment failed in only 19.8% of the programs. For 32.1% of the programs, no information is available to determine whether or not the pathogen established in the new area. Among those releases for which the fate was recorded, 70.8% became established while 29.2% did not.

4. Number of programs and rate of establishment by

4.1. Time

The earliest introductions we found recorded occurred in 1894–1895, when the fungal pathogen *Beauveria brongniartii* (Saccardo) Petch was released against scarab grubs in Australia. Thereafter, very few introduction programs were conducted worldwide each decade until about the 1950s (Fig. 1A). Throughout this time, only fungal pathogens were introduced and most introductions were made against various hemipteran species (65.5%) and scarabs (27.6%).

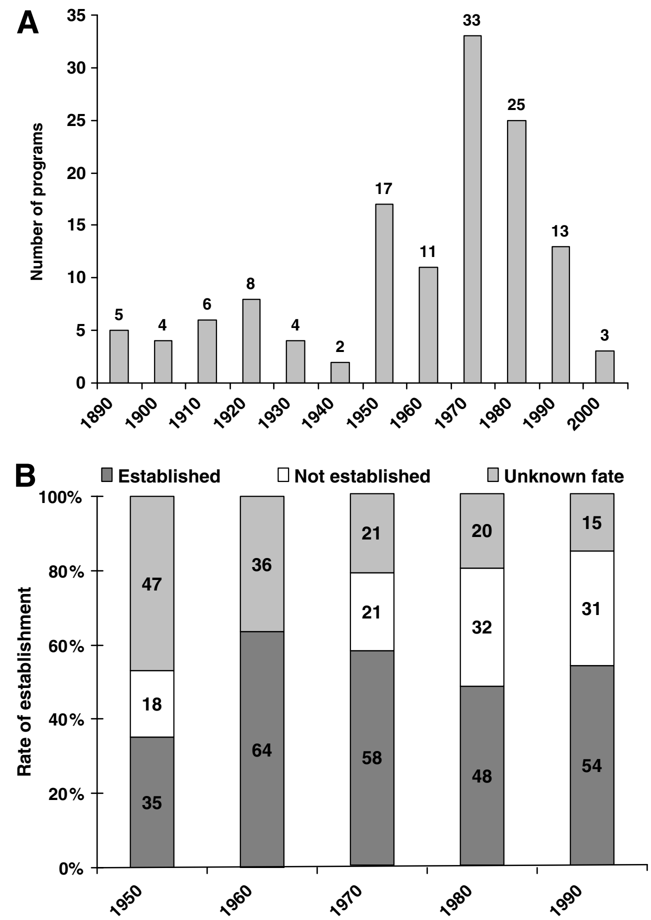

Fig. 1. (A) Numbers of classical biological control programs introducing arthropod pathogens and arthropod-parasitic nematodes by decade. Programs spanning more than one decade are counted in the decade when the program was initiated. (B) Rates of establishment of microbes and insect parasitic nematodes released for classical biological control by decade from 1950 to 1999. Earlier programs are not presented due to low numbers per decade and programs after 1999 are not included because we could not judge if enough time has passed since releases to evaluate establishment.

Beginning in the 1950s and in subsequent decades, a greater diversity of pathogens and nematodes was introduced and a greater diversity of insect pests was targeted by introductions. The greatest numbers of introductions occurred in the 1970s and 1980s, with this trend decreasing in the 1990s. The low number of introductions for the decade 2000–2009 in part reflects the fact that this decade is only partially complete at the time this paper is being published. However, this decrease could also have similar roots as the decline in classical biological programs introducing parasitoids and predators (e.g., Follett et al., 2000), that appear to be associated with increased regulatory restrictions to ensure environmental safety of releases.

For each decade, among the programs whose fate was recorded the majority resulted in establishment, with an average by decade of 72.8 ± 7.3% (mean ± SE) from 1950 through 1990 (Fig. 1B). However, the results from a significant number of programs during this period have not been reported (range: 15–47%), possibly because scientists were unable to return to sites to assess post-release establishment. The decade with the lowest establishment was 1910–1919; only 2 out of 6 releases resulted in establishment. During four decades 1890–1899, 1900–1909, 1910–1919 and 1960–1969, all introductions whose fate was reported resulted in establishment of the agents being released. Results from releases made in the decade beginning with 2000 are not included in Fig. 1B because insufficient time has passed to realistically evaluate establishment. However, of the three programs in this decade, we know that one of the releases yielded establishment (i.e., a release of *Entomophaga maimaiga* Humber, Shimazu and Soper into Bulgaria) while the other release programs cannot yet be evaluated.

4.2. Types of microbial natural enemies

Thirty-seven species of entomopathogens (i.e., viruses, bacteria, fungi, microsporidia and an oomycete) and eight species of insect parasitic nematodes have been used in classical biological control programs. Fungi were most often used followed by viruses and nematodes while other groups were seldom deployed (Fig. 2A). Most (91%) virus release programs resulted in establishment while establishment rates for fungi, microsporidia and nematodes were similar (33–41%) (Fig. 2B). Only one of the few bacterial release programs resulted in documented establishment. Differences in the percent of programs yielding establishment were observed not only among pathogen or nematode groups as a whole but also variability was seen among species within some groups.

The five species most frequently used in classical biocontrol programs were the *O. rhinoceros* virus (OrV) (= *Rhabdionvirus oryctes* (Huger); = *Baculovirus oryctes* Huger) ($n = 18$ releases), the fungal pathogens *Metarhizium anisopliae* (Metschnikoff) Sorokin ($n = 13$) and *E. maimaiga* ($n = 7$) and the nematodes *R. culicivorax* Ross and Smith (= *Reesimermis nielseni* Tsai and Grundmann) ($n = 11$) and *Deladenus* (=*Beddingia*) *siricidicola* Bedding ($n = 7$). The *O. rhinoceros* virus was by far the most successful microbe used in classical biological control programs because this virus became established every place it was released where post-release reports were available (i.e., results from releases in Guadalcanal, Solomon Islands could not be found). Another very successful example of classical biological control is the nematode *Deladenus siricidicola* which has been released for control of the woodwasp *Sirex noctilio* F. in several countries in the southern hemisphere. This nematode has been established in New Zealand, Australia, Brazil, Uruguay, South Africa and Argentina.

One of the most commonly released species, the fungal pathogen *M. anisopliae* (13 release programs), was released four times against *O. rhinoceros*. Most of the *M. anisopliae* programs were conducted at the beginning of the last century and only three programs have been undertaken during the last 30 years. This pathogen (like many anamorphic forms of fungi in the ascomycete Order Hypocreales, previously listed in the Class Hyphomycetes) has been used more recently in inundative augmentation approaches because experience has shown that this species is less likely to keep insect populations below the economic injury level when used for classical biological control and this species is amenable to mass production (Fuxa, 1987). Although *M. anisopliae* was one of the pathogens used more frequently in earlier years, in 84.6% of these early release programs the results of the releases are not available. In only two programs, the fungus persisted in soil and was recovered at least three years later, although infection prevalence was low. *M. anisopliae* is a cosmopolitan pathogen, found worldwide in many types of habitats, and strains of *M. anisopliae* from different geographic areas can vary in host range (Roberts and St. Leger, 2004). This species is also a facultative saprophyte. Because strains of this pathogen cannot be separated by morphology or vegetative and reproductive developmental patterns, they have to be identified and characterized biochemically at the strain level before importation in order to determine whether introduced strains have become established. Considering that

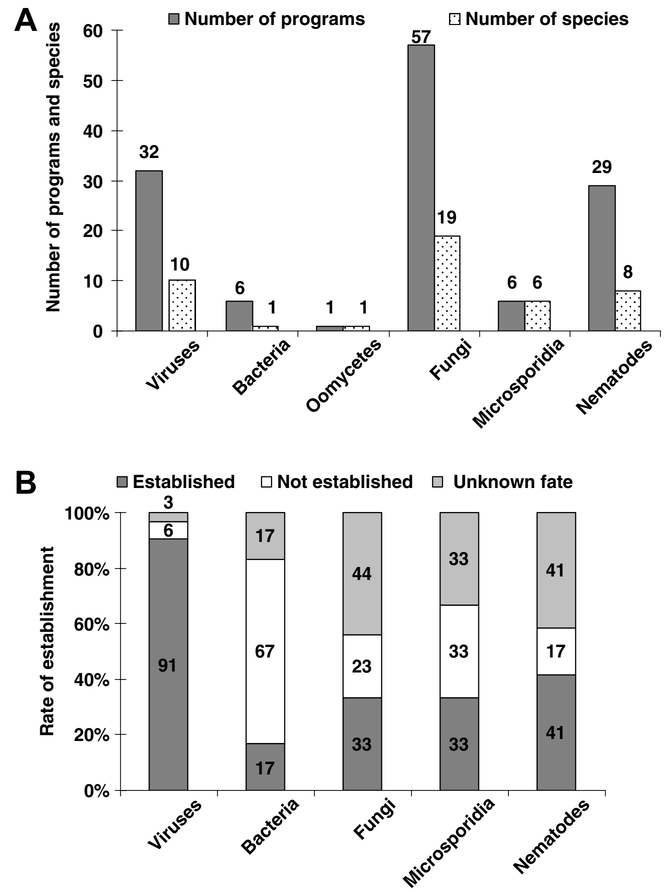

Fig. 2. (A) Numbers of programs and numbers of species from different groups of entomopathogens and insect parasitic nematodes released for classical biological control of arthropod pests. (B) Rates of establishment of microbes and insect parasitic nematodes released for classical biological control of arthropod pests, by pathogen group.

the introduced *M. anisopliae* strains were not characterized for any classical biological control introductions and the fact that endemic strains could potentially have been present before releases, it is perhaps unlikely that the true results of these releases will ever be known.

Nineteen species of entomopathogenic fungi (not including microsporidia) were released in 57 programs. In most of these programs, anamorphs of species of Ascomycetes in the Order Hypocreales were used, but in addition, seven species in the Order Entomophthorales were released in 17 areas. Although the fungi were the most widely used pathogens, they became established in only 33.3% of the programs. Numerically more Ascomycetes became established (13 of 40 programs, 36.1%) than Entomophthoraleans (4 of 17 programs, 23.5%), although percentages were not significantly different (Fisher's exact test; $P = 0.7524$). Among programs for which results are known, the entomophthoraleans failed to establish in 53.0% of the introductions compared to only 11.1% of Ascomycetes. Entomophthoraleans possess good attributes as classical biological control agents because of their strict host specificity, ability to cause epizootics and specialized resting spores for persistence. However, they are all obligate pathogens, meaning that they only proliferate and produce resting spores in nature after attacking living hosts. They are not easily mass produced, so in some programs low densities of inoculum were released (usually infected hosts). Entomophthoraleans have an intimate association with their hosts and an understanding of their life cycles is fundamental to improving the success and predictability of classical biological control (e.g., which environmental conditions induce resting spore formation, persistence and germination). In contrast, because of the broader host range of the anamorphs of Ascomycetes that have been used, such as *Beauveria bassiana* and *M. anisopliae*, these species have higher chances of contacting a susceptible host in the area of release. Some of these more generalist fungi can also persist saprotrophically. The ability of fungi to survive for prolonged periods in the absence of hosts has important implications for establishment and persistence.

Eight species of nematodes were released in 29 programs. Different species of nematodes varied greatly in their ability to establish. The nematode *D. siricidicola* released against *S. noctilio* established in all countries where it has been released, whereas the rate of establishment of other nematodes was only 22.7%. The nematode most commonly released, *R. culicivorax*, established in only four of 11 release programs, although results from five of the 11 programs are not known.

Ten species of viruses were introduced in 32 programs, with an overall establishment rate of 91.6% (Fig. 2B). Viruses are by far the most successful group of microbial natural enemies in terms of rate of establishment, regardless of the type of virus, which included non-occluded viruses as well as nucleopolyhedroviruses (NPVs) and granuloviruses (GVs).

Six species of microsporidia were released but only two established. Releases of bacteria established in only one of six programs. The only bacterial species used as a classical biological control agent was *P. popilliae*, released in Kiribati, Palau, American Samoa, Kenya, Tanzania and the Azores against scarab beetles. The oomycete, *Lagenidium giganteum* Couch, was the only pathogen within the Kingdom Chromista (= Kingdom Stramenopila) used for this control approach and the only release program resulted in establishment.

4.3. Types of hosts

The greatest numbers of host species or groups of host species targeted belong to the arthropod orders Diptera, Coleoptera and Hemiptera, followed by the Orthoptera and then Lepidoptera (Fig. 3A). The diversity of host species targeted from other arthropod orders was minimal, including one species of thrips and three species of mites. Only four species of hymenopterans were targeted, but some of these programs were extremely successful in

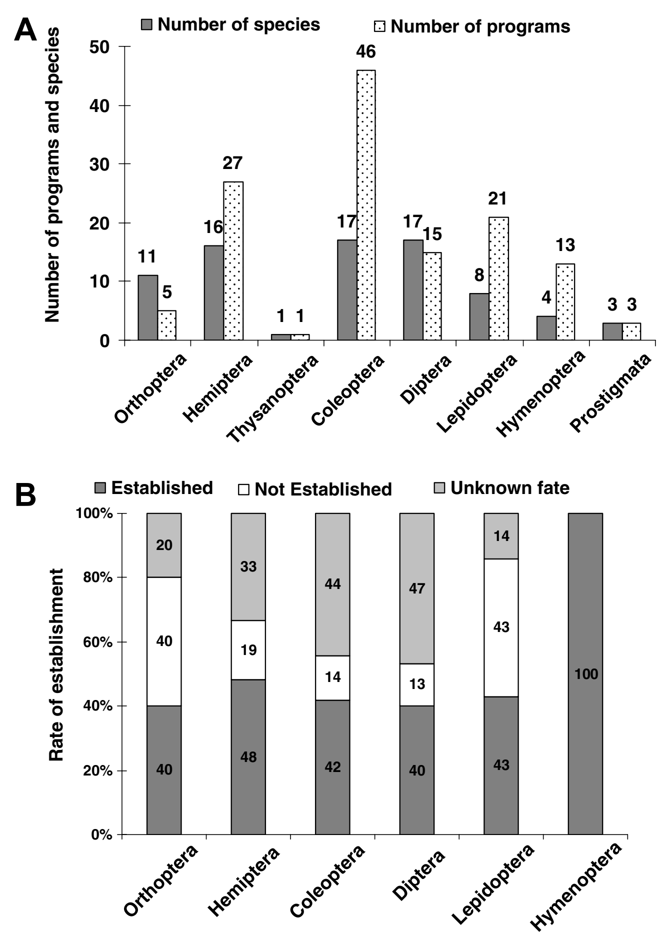

Fig. 3. (A) Numbers of programs and numbers of species from different groups of arthropod hosts targeted by classical biological control introductions of microbes and nematodes. (B) Rates of establishment of microbes and insect parasitic nematodes released for classical biological control of arthropod pests, by host order.

establishment and control and consequently these microbes were released in numerous areas, e.g., *D. siricidicola* against *S. noctilio*. Releases against two species of diprionid sawflies had an impact over large areas, e.g., NPVs released against European spruce sawfly, *Gilpinia hercyniae* (Hartig) and European pine sawfly, *Neodiprion sertifer* (Geoffrey).

Most releases (84.7%) targeted a single insect or mite species; however, some classical biological control programs were aimed at controlling groups of pests such as several species of white grubs in sugar cane, aphids in cereals, melanoplinae grasshoppers and anopheline species. The programs most commonly directed against a group of species were directed against mosquitoes; 8 out of 11 releases against mosquitoes were directed against more than one species of host.

Release programs always targeted only one insect order and the order with the most release programs was the Coleoptera, in part due to the many releases programs to control rhinoceros beetles *Oryctes* spp. in different areas (Hajek et al., 2005) (Fig. 3A). Release programs targeting Hemiptera and Lepidoptera were also relatively common, while fewer release programs were directed against Diptera, Hymenoptera and Orthoptera.

There was not much difference in the rate of establishment of releases conducted against groups of arthropod pests (40.0–48.1% established) with the exception of programs directed against hymenopteran pests (Fig. 3B) for which 100% success in establishment occurred. Almost equal numbers of programs targeted species of sawflies in the families Siricidae and Diprionidae. Programs against siricids were directed against *S. noctilio* using the nematode *D. siricidicola* while those against diprionids targeted two forest defoliators, *G. hercyniae* and *N. sertifer* using viruses specific to these species. The insect group with the greatest number of releases, the Coleoptera, had a high level of unknown results (44.4% of programs), although such a high percentage for programs in which results are not known was not unusual.

For the majority (63.6%) of release programs for which we could ascertain the area of endemism of the pest(s) and whether or not the released organisms became established, the targeted pest species was invasive in the release area and not native. The percentage of programs yielding successful establishment was not significantly different between those programs targeting native pests (71.4% establishment) versus invasive pests (72.4%) ($\chi^2 = 0.0091$; $P = 0.9240$).

4.4. Program locations

Classical biological control programs have been conducted in 49 different countries or island/island groups and on all continents except Antarctica. More introductions were conducted in North America ($n = 46$) than in other regions (Fig. 4), with the largest number occurring in the United States ($n = 33$).

A high percentage of the total release programs were conducted on islands (48.9%), most of which were oceanic.

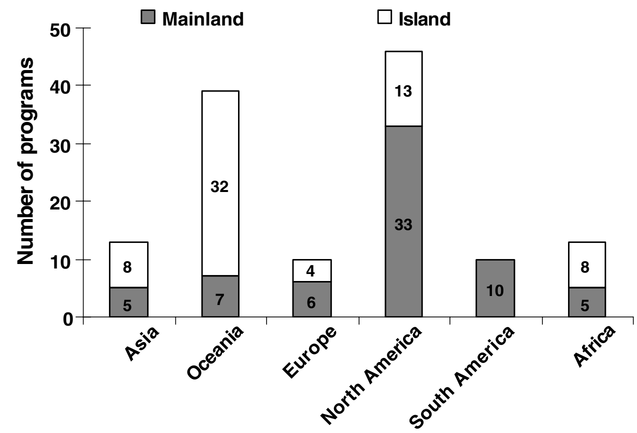

Fig. 4. Numbers of classical biological control programs introducing pathogens and nematodes for control of arthropods in mainland (i.e., continents) and islands or island groups (predominantly oceanic islands).

Percentages of establishment from releases on islands (48.4%) were not significantly different from establishment from releases in mainland areas (50.8%) ($\chi^2 = 0.0698$; $P = 0.7916$).

Many factors affect success in establishment of microbes used for classical biological control, including habitat type. However, it was not always possible to determine from the literature exactly what type of ecological habitat was associated with each program. Seventy-five of the 131 release programs (57.3%) targeted species of arthropod pests associated with forest trees or trees grown for crop production (woody perennials including palms) (Fig. 5A). Programs targeting pests living in forest trees and trees grown for crops had higher levels of success in establishment (>60%) compared with those programs targeting pests in all other types of habitats (⩽40%) (Fig. 5B). Success of classical biological control programs using parasitoids and predators has also been higher when introductions were against pests of perennial crops (e.g., forestry, orchards), as compared to pests of annual crops. Symondson et al. (2002) suggested that the transitory nature of annual crops, periodically disrupted by cultivation, pesticides, and crop rotations, deters the development of continuous predator–prey relationships and limits the number and diversity of natural enemies. This may help to explain the higher establishment rates of microbes and insect parasitic nematodes in more stable habitats. In arable ecosystems, establishment might also be affected by applications of fungicides and herbicides and cultivation practices that cause a reduction of infective or resting stages of microorganisms and nematodes in the soil. However, there are also examples where successful establishment and long-term control has been achieved in annual crops, e.g., introductions of *Nosema pyrausta* (Paillot) against European corn borer (*Ostrinia nubilalis* (Hübner)) in the United States. AgMNPV, successfully introduced against velvetbean caterpillar (*Anticarsia gemmatalis* (Hübner)) in soybeans, has a combination of characteristics well suited to the annual crop habitat. This NPV can persist for years in the soil,

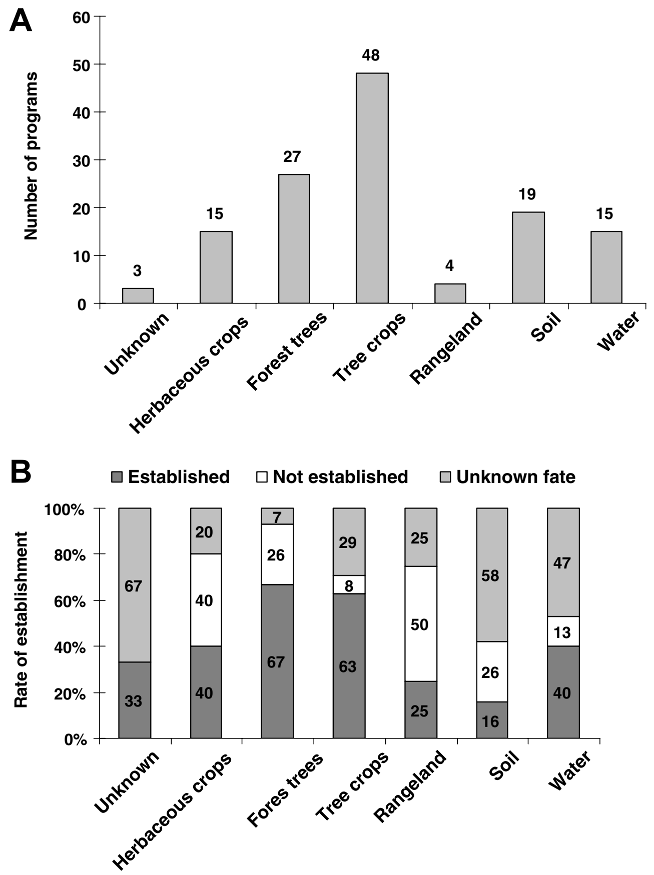

Fig. 5. (A) Numbers of classical biological control programs targeting arthropod pests in different types of habitats using microbes and nematodes. (B) Rates of establishment of microbes and insect parasitic nematodes released for classical biological control of arthropod pests, by habitat type. Herbaceous crops include annuals such as tobacco and soybeans, semi-perennials such as sugarcane and perennials such as alfalfa Tree crops are woody plants from which products are harvested and include standard orchard crops, coffee, palm plantations and vineyards.

can replicate rapidly, is rapidly spread by predators and is independent of host density for certain time periods (Fuxa and Richter, 1999).

5. Accidental introductions

The catalogue lists eight instances in which microbes or nematodes attacking insects were found in new areas without having been intentionally introduced. From data in the catalogue, four of these introductions were baculoviruses that infect larvae of two species of Lepidoptera and one species of diprionid. Two microsporidian species were found, one infecting fire ants (*Solenopsis invicta* Buren) in Louisiana and the other infecting the European corn borer. One nematode, *D. siricidicola*, was found infecting *S. noctilio* on the North Island of New Zealand and is assumed to have come from Europe. The fungus *E. maimaiga* was probably accidentally introduced into the US prior to its detection in 1989 (see Nielsen et al., 2005). A high percentage of species that are introduced into new locations either do not become established (see Williamson, 1996) or become established but probably are not detected for many years. In fact, we do not know how or when these species were actually introduced, rather only when they were first detected. It has been hypothesized that the three viruses and the one microsporidium were introduced inadvertently along with parasitoids that had been introduced for classical biological control (see Hajek et al., 2005).

6. Comparisons of patterns of establishment of macro and micro natural enemies

The implementation of classical biological control programs to control arthropod pests has predominantly involved the introduction of predators and parasitoids (= macro natural enemies); in comparison, arthropod pathogens and insect parasitic nematodes (= micro natural enemies) have seldom been utilized. The BIOCAT database (December 2005) presently lists 5670 introductions of parasitoids and predators for control of insect pests, with 2008 (35%) becoming established (D.J. Greathead, pers. comm.) (Table 1). In contrast, we documented only 131 introductions of pathogens and nematodes, although a high percentage of releases have yielded establishment (48.1% of total programs, or 70.8% of releases for which the fate is known). A few of these programs involving arthropod pathogens and nematodes have been exceptionally successful, resulting not only in establishment but also in control of the target pest; consequently the same organism was then introduced repeatedly at different locations. For example, the *Oryctes* non-occluded virus was found to provide control of *O. rhinoceros* at two locations in 1967 and was then released at 16 additional locations over a period of 21 years (until 1988). The exotic nematode, *D. siricidicola*, which was discovered providing excellent control of *S. noctilio* on the North Island of New Zealand in 1962, was subsequently introduced to seven geographic locations on four continents (the South Island of New Zealand, Australia, South Africa and southern South America) over a 32 year period.

Predators and parasitoids have been introduced much more extensively than pathogens and nematodes but trends in the use of these different natural enemies are evident even in past centuries (Table 1). From 1900 to 1909, there were >150 introductions of parasitoids and predators against insect pests (Greathead and Greathead, 1992) while there were only four introductions of pathogens during this period. The numbers of macro natural enemies introduced was never less than 100 per decade in subsequent decades, increasing to over 800 from 1960 to 1969, while introductions of pathogens and nematodes reached a maximum of only 31 in 1970–1979. Interestingly, there is no evidence of a trend in the level of success in establishment of parasitoids/predators or pathogens/nematodes over time. However, it has been suggested that in recent years fewer parasitoids and predators are being released but with more attention to studying each species before release, so that

Table 1
Comparison of results of classical biological control programs releasing macro (parasitoids and predators) versus micro (pathogens and nematodes) natural enemies

	Parasitoids and predators	Pathogens and nematodes[a]
No. of programs[b]	5670	131
No. and % of establishments[c]	2008 (35.4%)	63 (48.1%)
No. pest species	601	76
No. agent species	2130	45
No. countries/islands[d]	220	49
No. programs over time[e]	Began increasing the 1920s, with peak numbers of programs 1930–1939, 1950–1979	Began increasing in the 1950s, with peak numbers of programs 1970–1989
Target pests[e]	Most commonly Homoptera, followed by Lepidoptera, Coleoptera and then Diptera	Most commonly Coleoptera, followed by Hemiptera and then Lepidoptera
Diversity of countries[e]	Worldwide, most releases in the US	Worldwide, most releases in the US
Islands versus mainland[e]	Possible that success in control is greater on islands but not establishment	Similar establishment in each

Comparisons are made between a summary of introductions of insect natural enemies against insect pests in the BIOCAT database (as of December 2005, D.J. Greathead, February 2006, pers. comm.) and this paper.

[a] Including three programs, where mites were the target. The Greathead BIOCAT database only includes programs against insect pests.

[b] In this paper, the term 'program' refers to release of one species of agent in one geographic area although this would be called 'introduction' by Dr. Greathead, and the term 'program' with reference to the BIOCAT database can refer to release of several different species of natural enemies.

[c] Excluding both organisms that failed to establish and those where results were not known.

[d] Data for pathogens and nematodes could be a slight underestimate compared with data on parasitoids and predators because the former were analyzed by island group and the latter were often counted by individual island on which releases were made.

[e] Information is from the Greathead and Greathead (1992) summary of the BIOCAT database.

only those most promising for providing control and those most environmentally safe are released. These practices should increase the level of success.

Introductions of parasitoids and predators have been more common by far against hemipterans (Greathead and Greathead, 1992), many of which were invasive species belonging to the Suborder Homoptera (Greathead, 1989). Numerous introductions of parasitoids and predators have also been made against Lepidoptera, Coleoptera and Diptera, in that order. In contrast, programs for release of pathogens and nematode primarily targeted Coleoptera, followed by Hemiptera, Lepidoptera, Diptera and then Hymenoptera (Fig. 3A). In contrast to introductions of parasitoid and predators, when microbes and nematodes have not become established, frequently they were not released again at the same location, or at least subsequent attempts to introduce them were not documented. With releases of parasitoids and predators, it is not uncommon that releases not yielding establishment are tried again, under the hypothesis that the species could potentially be effective at that site but by chance previous releases were ill-fated. Programs introducing pathogens and nematodes also frequently targeted invasive species, as has also been the trend with introductions of parasitoids and predators (van Driesche and Bellows, 1996; Hajek, 2004).

Analyses of introductions of parasitoids and predators against arthropod pests have evaluated success of releases (e.g., Greathead and Greathead, 1992). We did not attempt to include the success at control in our analysis because it seemed that ratings regarding levels of control of target pests were often subjective and not readily comparable among different programs. Furthermore, most of the literature used in the catalogue did not provide clear summaries of results. Problems with defining and monitoring control success are well known and universal, which may explain the apparently poor success rates for global efforts in biological control (Fowler, 2000). The criteria for assessing success of pathogens and nematodes in classical biological control programs differ significantly from what has been proposed for biological control of weeds and biological control of arthropods using parasitoids and predators. Whereas epizootics of some pathogens such as entomophthoralean fungi and NPVs can have remarkable impacts and cause high mortality in high density host populations, microbes such as microsporidia and some nematodes can debilitate host population over time or cause chronic effects. For example, Zelinskaya (1980) reported that microsporidian infections in European gypsy moth (*Lymantria dispar*) populations caused decreased fecundity, increased numbers of unfertilized eggs, increased overwintering mortality of embryos, and high mortality of maternally infected early stage larvae. Microsporidia-infected gypsy moth larvae also have slower development rates which may increase their susceptibility to stage-specific parasitoids. These effects are more difficult to document but can have a significant impact on target host dynamics. In agreement, Fuxa (1987) suggested that the greatest potential for utilizing entomopathogens in IPM might be realized through their inoculative augmentation and introduction for establishment, including introduction of new strains of pathogen species already present. These approaches minimize certain entomopathogen weaknesses such as slow debilitation of pest individuals and populations while taking advantage of ecological strengths such as recycling, persistence, and rapid generation times.

Some of the life history characteristics that have been considered optimal for the successful use of species of parasitoids and predators in classical biological control (Hajek, 2004) also are important in the success of introduced pathogens and nematodes. Most species of pathogens and nematodes that have been successfully established after release and have been documented to provide control possess horizontally transmitted stages that are environmentally resistant, thus providing excellent persistence. Many successful pathogens and nematodes are also virulent, at times causing high levels of mortality. Such attributes agree with predictions by Anderson and May (1980) that virulence and persistence are requirements for long-lasting cyclic control. Fuxa (1987) also noted that the rapid generation time of pathogens is an ecological strength that adapts these organisms for control. However, some studies have refuted the connection between rapid host mortality (= high virulence) and successful colonization and persistence (e.g., Lee et al., 2001; Sun et al., 2003). Successful species are often highly host specific or at least have narrow host ranges. Also, success is usually associated with pathogen and nematode species obtained from areas similar in climate to areas where they are to be released. Notable examples of this are the fungal pathogen *E. maimaiga* that attacks gypsy moth, the spotted alfalfa aphid pathogen *Z. radicans*, the NPVs released against European spruce and pine sawflies, the non-occluded virus released against palm rhinoceros beetles, and the nematodes *D. siricidicola* released against *S. noctilio* and *Steinernema scapterisci* Nguyen and Smart released against mole crickets.

7. The role of regulations

Why are the numbers of programs introducing pathogens and nematodes against arthropod pests so few compared with programs releasing parasitoids and predators? Milner (1986) suggested that there are a small number of opportunities for introducing pathogens and nematodes, especially because the most effective insect pathogens already occur worldwide, either naturally or because they have been accidentally introduced with their hosts. However, our list of accidental introductions is very short, which does not support this suggestion. We hypothesize that classical biological control programs have primarily focused on collecting and introducing predators and parasitoids because these have historically been recognized as important natural enemies by entomologists and are larger and easier to recover and manipulate. Maddox et al. (1992) suggested that there are several reasons why entomopathogens have not been used as classical biological control agents, foremost being that the diagnosis, isolation and culture of entomopathogens required in foreign exploration programs requires more specialized procedures and equipment than is needed for collecting and rearing most parasitoids and predators. For example, foreign exploration for exotic microsporidia infecting native gypsy moth populations in Europe sometimes required individual dissection of specific tissues from thousands of larvae, frequently without success (McManus, unpublished data). Additionally, the taxonomy of many groups of entomopathogens is ambiguous and important biological characteristics such as host specificity and ecological interactions have not been determined for many species (Maddox et al., 1992).

Despite the reasons listed previously to explain why entomopathogens have been used infrequently in classical biological control, it is important to also consider the role of regulations or the lack thereof and their impact on past and future programs. With the emergence in the last century of Integrated Pest Management (IPM) and the emphasis placed on introduced (exotic) biological control agents as a major component of IPM, practioners became concerned about the potential danger of these agents on indigenous flora and fauna, particularly on sensitive and endangered species. Countries with little or no experience in biological control began introducing exotic biological control agents, which prompted organizations such as the International Organization for Biological Control (IOBC) to approach the Food and Agricultural Organization (FAO) about developing a "Code of Conduct" to provide guidance for future introductions (Greathead, 1997). In response to these concerns, in 1995, FAO endorsed the Third International Standard for Phytosanitary Measures, ISPM 3: code of conduct for the import and release of exotic biological control agents (FAO/IPPC, 1996).

The situation in the US for importation and release of biological control agents was more complex (Hajek et al., 2000). Insect parasitic nematodes were not regulated by the Environmental Protection Agency (EPA), while other microbes were regulated by the EPA as if they were pesticides (i.e., toxicity testing was required, although to a lesser extent than required for synthetic chemical pesticides), even if the intent was to use these organisms for classical biological control. This policy regarding microbes was viewed as a deterrent to potential use of entomopathogens in biological control programs and prompted Miller and Aplet (1993) to conclude that specific legislation was needed to develop guidelines and provide a roadmap for the safe use of biological control agents.

Fortunately, the impasse was broken when, in 2000, the North American Plant Protection Organization (NAPPO), a plant protection organization created in 1976 to coordinate the efforts of Canada, the US and Mexico under the authority of FAO, endorsed a standard similar to that endorsed by the European Plant Protection Organization (EPPO) earlier in the same year (EPPO, 2000). Both of these regional organizations used the FAO Code of Conduct (FAO/IPPC, 1996) as their baseline document. APHIS, the US representative to NAPPO, then provided a directive for requests to release non-native entomopathogens and insect parasitic nematodes for biological control of pest insects and mites. These guidelines are intended to assist researchers in drafting a petition for release of exotic entomophagous agents for biological control and to assist reviewers and regulators in assessing the risks and benefits of introductions.

Each US permit application (PPQ Form 526) to request permission to release a non-native entomopathogen should include the following broad categories of information, each in the form of a separate report: proposed action; biological control agent information; target pest information; environmental and economic impacts of the proposed release. There are 5–10 specific requests for information within each of these broad categories. The details for entomopathogen permit applicants can be accessed at the website: http://www.aphis.usda.gov/ppq/permits/biological/entomopathogens.html. A formal review of proposals requesting the release of entomopathogens is facilitated through NAPPO by APHIS-PPQ, Pest Permits Evaluation Branch.

The development of standards for the introduction of exotic biological control in the US has been a painfully slow process. Hopefully, the preparation and publication of these guidelines will encourage scientists to consider the use of entomopathogens as classical biological control agents in future IPM programs.

8. Environmental concerns of deliberate introduction of exotic species

There is still controversy surrounding the deliberate introduction of exotic species for biological control and whether or not the benefits of these actions will outweigh the environmental costs. Critics state that predicting the outcomes of such introductions is infinitely more complex than simply stating that introductions of exotic biological control agents are needed to impart balance to populations of established invasive non-native species (Hoddle, 2004). Louda and Stiling (2004) suggested that before exotic biological control agents are introduced, studies are needed to assess the full complement of their interactions in a new environment.

No documented case was found in the literature used in the catalogue where a pathogen introduced for classical biological control of an insect pest caused substantial mortality to a non-target species or caused negative effects to human and animal health, or any other significant impact to the environment. The environmental impacts and non-target effects of the introductions of different groups of arthropod pathogens and insect parasitic nematodes have been investigated by Fuxa (1989), Laird et al. (1990) and Hokkanen and Hajek (2003). These authors have concluded that these natural enemies have been safe and environmentally benign, although it is recommended that the impact of new introductions must be evaluated on a case by case basis.

Mistakes have been made in the past, in particular where multiple species of biological control agents have been introduced and where prior adequate biological (i.e., host specificity) and environmental assessments were not conducted. Denoth et al. (2002) suggested that in the past, multiple agents have been released not to increase the cumulative impact on a target host, but rather to increase the likelihood that the right control species was released. This reasoning is no longer acceptable and, in accordance, procedures have changed. The International Standards that were established by FAO/IPPC (1996) and that precipitated the guidelines published by APHIS for the importation of entomopathogens for small-scale (<4 ha) experimental purposes should address the concerns of the environmental community and at the same time, provide a roadmap for scientists interested in pursuing the introduction of entomopathogens for classical biological control.

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References

Anderson, R.M., May, R.M., 1980. Infectious diseases and population cycles of forest insects. Science 210, 658–661.

Baird, R.B., 1958. The artificial control of insects by means of entomogenous fungi: a compilation of references with abstracts. Canadian Agricultural Research Station, Kentrille, Nova Scotia, Canada, 53 pp.

Berger, E.W., 1906. Whitefly conditions in 1906 – the use of the fungi. Fla. Agric. Exp. Sta. Bull. 88, 51–85.

Burges, H.D., 1981. Strategy for the microbial control of pests in 1980 and beyond. In: Burges, H.D. (Ed.), Microbial Control of Pests and Plant Diseases 1970–1980. Academic Press, London, pp. 797–836.

Burges, H.D., Hussey, N.W., 1971. Past achievements and future prospects. In: Burges, H.D., Hussey, N.W. (Eds.), Microbial Control of Pests and Plant Diseases. Academic Press, New York, pp. 687–709.

Caltagirone, L.E., Doutt, R.L., 1989. The history of the vedalia beetle importation to California and its impact on the development of biological control. Annu. Rev. Entomol. 34, 1–16.

Carruthers, R.I., Ramos, M.E., Larkin, T.S., Hostetter, D.L., Soper, R.S., 1996. The *Entomophaga grylli* (Fresenius) Batko species complex: its biology, ecology, and use for biological control of pest grasshoppers. Mem. Entomol. Soc. Can. 171, 329–353.

Clausen, C.P. (Ed.), 1978. Introduced Parasites and Predators of Arthropod Pests and Weeds: A World Review. USDA, Agriculture Handbook No. 480, Washington, DC.

DeBach, P., 1974. Biological Control by Natural Enemies. Cambridge University Press, London, UK.

Denoth, M., Frid, L., Myers, H., 2002. Multiple agents in biological control: improving the odds? Biol. Control 24, 20–30.

Dustan, A.G., 1923. Studies on a new species of *Empusa* parasitic on the green apple bug (*Lygus communis* var. *novascotiensis* Knight) in the Annapolis Valley. Proc. Acadian Entomol. Soc. 9, 14–36.

Dustan, A.G., 1924. The control of the European apple sucker, *Psylla mali* Schmidb., in Nova Scotia. Canada, Dept. Agric., Pamph. No. 45 (New Series). 13 pp.

Eilenberg, J., Hajek, A., Lomer, C., 2001. Suggestions for unifying the terminology in biological control. Biol. Control 46, 387–400.

EPPO (European and Mediterranean Plant Protection Organization), 2000. EPPO Standards, Import and Release of Exotic Biological

Control Agents. PM 6/2(1) English. European and Mediterranean Plant Protection Organization, Paris, France, 4 p.

FAO/IPPC (Food and Agriculture Organization, International Plant Protection Convention). 1996. Code of Conduct for the Import and Release of Exotic Biological Control Agents. ISPM No.3, IPPC Secretariat, FAO, Rome, Italy.

Follett, P.A., Duan, J., Messing, R.H., Jones, V.P., 2000. Parasitoid drift after biological control introductions: re-examining Pandora's box. Am. Entomol. 46, 82–94.

Fowler, S.V., 2000. Trivial and political reasons for the failure of classical biological control of weeds: a personal view. In: Spencer, N.R. (Ed.), Proc. X Int. Symp. Biol. Control Weeds 4–14 July 1999. Montana State University, Bozeman, MT, USA, pp. 169–172.

Fuxa, J.R., 1987. Ecological considerations for the use of entomopathogens in IPM. Annu. Rev. Entomol. 32, 225–251.

Fuxa, J.R., 1989. Fate of released entomopathogens with reference to risk assessment of genetically engineered microorganisms. Bull. Entomol. Soc. Am. 35, 12–24.

Fuxa, J.R., Richter, A.R., 1999. Classical biological control in an ephemeral crop habitat with *Anticarsia gemmatalis* nucleopolyhedrovirus. Biol. Control 44, 403–419.

Fuxa, J.R., Ayyappath, R., Goyer, R.A., 1998. Pathogens and microbial control of North American forest insect pests. USDA, Forest Service, FHTET-97-27.

Goettel, M.S., Hajek, A.E., 2001. Evaluation of non-target effects of pathogens used for management of arthropods. In: Wajnberg, E., Scott, J.K., Quimby, P.C. (Eds.), Evaluating Indirect Ecological Effects of Biological Control. CABI Publisher, Wallingford, Oxon, UK, pp. 81–97.

Gopal, M., Gupta, A., Sathiamma, B., Radhakrishnan Nair, C.P., 2001. Control of the coconut pest *Oryctes rhinoceros* L. using the *Oryctes* virus. Insect Sci. Appl. 21, 93–101.

Greathead, D.J., 1989. Biological control as an introduction phenomenon: a preliminary examination of programmes against Homoptera. Entomologist 108, 28–37.

Greathead, D.J., 1997. An introduction to the FAO code of conduct for the import and release of exotic biological control agents. Biocontrol News Inf. 18, 117N–118N.

Greathead, D.J., Greathead, A.H., 1992. Biological control of insect pests by insect parasitoids and predators: The BIOCAT database. Biocontrol News Inf. 13, 61N–68N.

Groves, R.H., 1986. Plant invasions of Australia: an overview. In: Groves, R.H., Burdon, J.J. (Eds.), Ecology of Biological Invasions: An Australian Perspective. Austral. Acad. Sci., Canberra, pp. 149–187.

Hajek, A.E., 2004. Natural Enemies: An Introduction to Biological Control. Cambridge University Press, Cambridge, UK.

Hajek, A.E., Delalibera Jr., I., McManus, M.L., 2000. Introduction of exotic pathogens and documentation of their establishment and impact. In: Lacey, L.A., Kaya, H.K. (Eds.), Field Manual of Techniques in Invertebrate Pathology. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 339–369.

Hajek, A.E., McManus, M.L., Delalibera, Jr., I., 2005. Catalogue of Introductions of Pathogens and Nematodes for Classical Biological Control of Insects and Mites. USDA, For. Serv. FHTET-2005-05.

Hall, I.M., Dunn, P.H., 1957a. Entomophthorous fungi parasitic on the spotted alfalfa aphid. Hilgardia 27 (4), 159–181.

Hall, I.M., Dunn, P.H., 1957b. Fungi on spotted alfalfa aphid. California Agric. 11 (2), 5.

Hall, I.M., Dunn, P.H., 1958. Artificial dissemination of entomophthorous fungi pathogenic to the spotted alfalfa aphid in California. J. Econ. Entomol. 51, 341–344.

Hoddle, M.S., 2004. Restoring balance: using exotic species to control invasive exotic species. Conservation Biol. 18, 38–49.

Hodge, K.T., Sawyer, A.J., Humber, R.A., 1995. RAPD-PCR for identification of *Zoophthora radicans* isolates in biological control of potato leafhopper. J. Invertebr. Pathol. 65, 1–9.

Hokkanen, H.M.T., Hajek, A.E. (Eds.), 2003. Environmental Impacts of Microbial Insecticides: Need and Methods for Risk Assessment. Kluwer Academic Publishers, Dordrecht, NL.

Julien, M.H., Griffiths, M.W. (Eds.), 1998. Biological Control of Weeds, A World Catalogue of Agents and their Target Weeds. CABI Publisher, Wallingford Oxon, UK.

Kaya, H.K., Stock, S.P., 1997. Techniques in insect nematology. In: Lacey, L.A. (Ed.), Manual of Techniques in Insect Pathology. Academic Press, San Diego, CA, pp. 281–324.

Keane, R.M., Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol. Evol. 17, 164–170.

Laird, M., Lacey, L.A., Davidson, E.W., 1990. Safety of Microbial Insecticides. CRC Press, Boca Raton, FL.

Lee, Y., Fuxa, J.R., Inceoglu, A.B., Alaniz, S.A., Richter, A.R., Reilly, L.M., Hammock, B.D., 2001. Competition between wild-type and recombinant nucleopolyhedroviruses in a greenhouse microcosm. Biol. Control 20, 84–93.

Louda, S.M., Stiling, P., 2004. The double-edged sword of biological control in conservation and restoration. Conservation Biol. 18, 50–53.

MacLeod, D.M., 1963. Entomophthorales infections. In: Steinhaus, E.A. (Ed.), . Insect Pathology: An Advanced Treatise, vol. 2. Academic Press, New York, pp. 189–231.

Maddox, J.V., McManus, M.L., Jeffords, M.R., Webb, R.E., 1992. Exotic insect pathogens as classical biological control agents with an emphasis on regulatory considerations. In: Kauffman, W.C., Nechols, J.E. (Eds.), Selection Criteria and Ecological Consequences of Importing Natural Enemies. Entomol. Soc. Am., Lanham, MD, pp. 27–39.

Mendes, C., Lacey, L., Amaral, J., Klein, M., 1994. Biological control of *Popillia japonica* on Terceira Island (Azores, Portugal): potential of *Bacillus popilliae*. Bull. IOBC/WPRS 17 (3), 31–34.

Miller, M., Aplet, G., 1993. Biological control: a little knowledge is a dangerous thing. Rutgers Law Rev. 45, 285–334.

Milner, R.J., 1986. Pathogen importation for biological control – risks and benefits. In: Gibbs, A.J., Meischke, H.R.C. (Eds.), Pests and Parasites as Migrants. Cambridge University Press, Cambridge, UK, pp. 115–121.

Nielsen, C., Milgroom, M.G., Hajek, A.E., 2005. Genetic diversity in the gypsy moth fungal pathogen *Entomophaga maimaiga* from founder populations in North America and source populations in Asia. Mycol. Res. 109, 941–950.

Osborne, L.S., Landa, Z., 1992. Biological control of whiteflies with entomopathogenic fungi. Fla. Entomol. 75, 456–471.

Pimentel, D. (Ed.), 2002. Biological Invasions, Economic and Environmental Costs of Alien Plant, Animal, and Microbe Species. Springer, Berlin.

Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'Connell, C., Wong, E., Russel, L., Zern, J., Aquino, T., Tsomondo, T., 2000. Economic and environmental threats of alien plant, animal, and microbe invasions. Agric. Ecosys. Environ. 84, 1–20.

Roberts, D.W., St. Leger, R.J., 2004. *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: mycological aspects. Adv. Appl. Microbiol. 54, 1–70.

Sheppard, A.W., 1992. Predicting biological weed control. VII. International Symposium on Biological Control of Weeds, Lincoln, University New Zealand. Trends Ecol. Evol. 7 (9), 290–291.

Speare, A.T., Colley, R.H., 1912. The artificial use of the brown-tail fungus in Massachusetts, with practical suggestions for private experiment, and a brief note on a fungous disease of the gypsy caterpillar. Wright and Potter Printing Co., Boston.

Stiling, P., 1993. Why do natural enemies fail in classical biological control programs. Am. Entomol. 39, 31–37.

Sun, J., Fuxa, J.R., Henderson, G., 2003. Effects of virulence, sporulation, and temperature on *Metarhizium anisopliae* and *Beauveria bassiana* laboratory transmission in *Coptotermes formosanus*. J. Invertebr. Pathol. 84, 38–46.

Symondson, W.O.C., Sunderland, K.D., Greenstone, M.H., 2002. Can generalist predators be effective biocontrol agents? Annu. Rev. Entomol. 47, 561–594.

Tanada, Y., Kaya, H., 1993. Insect Pathology. Academic Press, San Diego, CA.

Theunis, W., Teuriara, N., 1998. Biological control of *Papuana huebneri* (Coleoptera, Scarabaeidae) in Kiribati: field trials with *Metarhizium anisopliae* and *Bacillus popilliae*. J. So. Pac. Agric. 15, 46–51.
van Driesche, R.G., Bellows Jr., T.S., 1996. Biological Control. Chapman & Hall, New York.
Williamson, M.H., 1996. Biological Invasions. Chapman & Hall, London.
Zelinskaya, L.M., 1980. Role of microsporidia in the abundance dynamics of the gypsy moth, *Porthetria dispar*, in forest plantings along the lower Dnieper river (Ukrainian Republic, USSR). Vestn. Zool. 1, 57–62.