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Environmental risk assessment of exotic natural enemies used in inundative biological control

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Abstract. In the past 100 years many exotic natural enemies have been imported, mass reared and released as biological control agents. Negative environmental effects of these releases have rarely been reported. The current popularity of inundative biological control may, however, result in problems, as an increasing number of activities will be executed by persons not trained in identification, evaluation and release of biological control agents. Therefore, a methodology for risk assessment has been developed within the EU-financed project 'Evaluating Environmental Risks of Biological Control Introductions into Europe [ERBIC]' as a basis for regulation of import and release of exotic natural enemies used in inundative forms of biological control (i.e. not in 'classical biological control' though some of the same principles and approaches apply). This paper proposes a general framework of a risk assessment methodology for biological control agents, integrating information on the potential of an agent to establish, its abilities to disperse, its host range, and its direct and indirect effects on non-targets. Of these parameters, estimating indirect effects on non-targets will be most difficult, as myriads of indirect effects may occur when generalist natural enemies are introduced. The parameter 'host range' forms a central element in the whole risk evaluation process, because lack of host specificity might lead to unacceptable risk if the agent establishes and disperses widely, whereas, in contrast, a monophagous biological control agent is not expected to create serious risk even when it establishes and disperses well. Drawing on published information and expert opinion, the proposed risk assessment methodology is applied to a number of biological control agents currently in use. These illustrative case histories indicate that the risk assessment methodology can discriminate between agents, with some species attaining low 'risk indices' and others scoring moderate or high. Risk indices should, however, not be seen as absolute values, but as indicators to which a judgement can be connected by biological control experts for granting permission to release or not.

Key words: direct effects, dispersal, environmental risk assessment, establishment, guidelines, host specificity, indirect effects, intraguild predation, non-target effects

1. Introduction

In the more than 100 years of biological control hundreds of species of exotic natural enemies have been imported, mass reared and released, resulting in successful control of many species of pests (e.g. Greathead, 1995; van Lenteren, 2000, 2003; Wratten and Gurr, 2000). Until now, very few problems have occurred concerning negative effects of these releases (e.g. Lynch and Thomas, 2000; Lockwood et al., 2001; Lynch et al., 2001). Examples of non-target effects put forward by Howarth (1985, 1991) have been seriously criticised by e.g. Follett et al. (2000), but several cases mentioned in the discussion section of this paper illustrate that release of exotics is not always without risk. That said, an important difference between biological control and the use of chemical pesticides is that natural enemies are often self-perpetuating and self-dispersing, and as a result biological control is regularly irreversible, although this is not always the case in inundative types of biological control. (Inundative biological control is the release of large numbers of mass-produced biological control agents to reduce a pest population without necessarily achieving continuing impact or establishment; classical biological control is the intentional introduction and permanent establishment of an exotic biological agent for long-term pest management). It is exactly the self-perpetuating, self-dispersing and irreversibility that is so highly valued in properly executed classical biological control programmes: it makes them sustainable and highly economic compared to any other control method (Bellows and Fisher, 1999; van Lenteren, 2001). The current popularity of commercial, inundative biological control may, however, result in problems, as an increasing number of activities will be executed by persons not trained in this field of pest control. One of these problems is the release of exotic natural enemy species that may cause negative effects on non-target species and the ecosystems in which these species function. Negative effects of insect biological control might be prevented when, like in modern-day weed biological control programmes (e.g. Wapshere, 1974; Blossey, 1995; Lonsdale et al., 2001), the effect is not only determined on the target species, but also on indigenous non-target species (van Lenteren, 1986, 1995; Blossey, 1995). Until relatively recently, testing of indigenous non-target species has rarely been applied as part of pre-release evaluation programmes for arthropod natural enemies (van Lenteren and Woets, 1988; Waage, 1997; Barratt et al., 1999). However, in classical biological control, there are several cases where non-target species testing has been applied properly. Examples are the programmes for control of *Sitonia* beetles in lucerne in New Zealand (e.g. Barratt et al., 1999) and that for control of cassava mealybugs in Africa (Neuenschwander and Markham, 2001). Another way to reduce the risks associated with the release of exotic natural enemies would be to limit the number of releases themselves by increasing the use of native natural enemies. Although this seems a logical approach, in the last four decades many exotic biological control agents were imported and released without thorough evaluation of the properties of native natural enemies that could have been candidates for inundative biological control (e.g. van Lenteren, 2000, 2003).

The potential risks of releases of exotic natural enemies have only recently received attention outside the biological control world, and an increasing number of countries now apply risk assessment procedures before a new natural enemy can be imported or released (for an overview, see OECD, 2003). Currently, about 25 countries are using some form of regulation concerning import of exotic biological control organisms. Some procedures (e.g. those of Australia, New Zealand and Hawaii; see articles in Lockwood et al., 2001) are already so strict that import and release of exotic natural enemies is extremely difficult. Other countries have no regulations at all, so any species can be imported and released. There is a general trend, however, towards more stringent regulatory requirements (e.g. Barratt et al., 1999). Implementation of regulation is considered by many countries, and is expected to significantly increase during the coming decade as a result of the agreements reached during the sixth UN-meeting of the Convention on Biological Diversity (Convention on Biological Diversity (CBD), 2002) as an approach to prevent the spread of invasive alien species.

Because of lack of in-depth ecological studies concerning mass-releases of exotic natural enemies, a four-year research project was initiated in 1998 on ‘Evaluating Environmental Risks of Biological Control Introductions into Europe’ [ERBIC], which is funded by the European Union 4th Framework Programme. To advance practical understanding of the environmental consequences of biological control and the ecological mechanisms involved, the ERBIC project adopted a three-pronged approach. First, as necessary background to the novel work done in the course of the project, a review of the known non-target effects of biological control world-wide was conducted (Lynch and Thomas, 2000). Secondly, four European biological control systems representing different agro-ecosystem contexts were taken as case studies, and subjected to various empirical investigations (for an overview of these case studies, see Lynch et al., 2001). Thirdly, ecological theory is being used as a conceptual basis for considering non-target risk, so as to unify these case studies within a broader framework in this way. In this paper, aspects of this work are presented and a risk assessment methodology appropriate for evaluation of inundative biological control is proposed. Because of its nature, the procedures for risk evaluation in classical biological control are likely to be more complex, though some of the mechanisms and approaches we identify will still apply.

The challenge in developing risk assessment methodologies is to develop protocols and guidelines that will prevent serious mistakes through import and release of harmful exotics, while at the same time still allowing safe forms of biological control to proceed. In this, the most critical ecological issues are to estimate the probabilities of attack on non-target organisms, and the dispersal and establishment capacities of the biological control agent. Few natural enemies are strictly monophagous (Zwölfer, 1971), but many are oligophagous and thus have a restricted host/prey choice. Sometimes the biological control industry favours the release of polyphagous natural enemies in order to be able to apply them for the control of various taxonomically unrelated pest species. These natural enemies in particular are expected to cause non-target effects.

A working group of the Organization for Economic Cooperation and Development (OECD), in collaboration with the EU-ERBIC project, is developing guidelines for harmonised information requirements for import and release of invertebrate biological control agents used in inundative types of biological control. These guidelines will be published in 2003 (OECD, 2003). This paper first presents a general framework for risk assessment procedures for biological control agents, and a more detailed framework, including methodology, for host-specificity testing and estimation of environmental risks. Then the proposed risk assessment procedure is applied to a number of biological control cases, and conclusions are drawn concerning the application of this methodology.

2. A general framework for regulation of import and release of biological control agents

In developing a general framework for regulation of import and release of biological control agents, the available codes of conduct and guidelines produced by various organisations and countries (e.g. FAO, EPPO, NAPPO, CABI, Austria, Australia, Czech Republic, Japan, Hungary, Norway, Sweden, Switzerland and New Zealand) were studied. However, most guidelines (with the exception of the ones from New Zealand (Barratt et al., 1999) and Australia (Paton, 1992)) are not very specific concerning criteria and methodology, so we decided to develop more specific guidelines including methodology and criteria based on work of the EU-ERBIC project. Regulation procedures for biological control agents will – like those for chemical pesticides – be characterised by questions concerning four issues:

1. Characterisation and identification of biological control agent: classical methods or molecular techniques, voucher specimens to be deposited, DNA fingerprinting in case of taxonomic problems;
2. Health risks: for arthropod natural enemies these will be much easier to determine than for chemical agents;
3. Environmental risks: see below;
4. Efficacy: efficacy of biological control agents can be highly variable, particularly if no proper mass rearing (van Lenteren and Tommasini, 2003) and quality control methods (van Lenteren, 2003) are applied. Efficacy is treated differently than in cases concerning chemical control. Because biological control agents often form part of an IPM programme, it is not necessary to reach 90–100% control by the biological control agent alone, as long as the total IPM programme results in sufficient reduction of pest or disease. Therefore, efficacy for biological control agents is defined as the ability to cause a significant reduction in number of pest organisms, direct and indirect crop damage, or yield loss.

The environmental risk assessment is the most critical and difficult part of the risk assessment procedure in biological control. Environmental assessments related to the release of exotic natural enemies are expected to contain the following two elements (based on activities of the OECD working group 'Guidelines for Registration Requirements for Invertebrate Biological Control Agents'): (1) identification of potential hazards posed to the environment based on collation of information, and data from experiments and observations, (2) a summary of the risks and benefits of the release of the exotic natural enemy in comparison with alternative control methods. Post-release reporting of any adverse effects on non-targets will be used to adjust environmental risk assessments and to decide about future releases in other areas. Most biological control projects include post-release studies to verify and monitor the establishment of a natural enemy (e.g. Cullen, 1997), but usually only the impact on the target species is studied. Barratt et al. (1999) propose to include non-target species in such follow up studies, because only then can the predictive value of pre-release risk evaluations be estimated and the testing methods improved. Based on post-release studies, Barratt et al. (1999) were able to compare the laboratory and field host ranges of two related parasitoids with large differences in their host ranges, and could conclude that laboratory measured host ranges (i.e. host-specificity testing, see below) were indeed indicative of field host ranges.

Below, we concentrate our discussion on the identification of potential hazards posed to the environment as a result of establishment, dispersal, host/prey range, and direct and indirect effects of release of the exotic natural enemy species. Our approach highlights the significance of these different factors and the ecological mechanisms involved, and it also provides some guidelines as to the data requirements and approaches that may be useful in their quantification. Information about these issues forms the basis of the environmental risk assessment, which we outline in the final part of the paper.

The terminology we use in this paper is based on existing key terms used in international instruments, relevant to invasive species, biodiversity, and pest control such as FAO (1999), OECD (2003) and Convention on Biological Diversity (2002); for definitions related to biological control, see Eilenberg et al. (2001).

3. Evaluation of the ecological factors determining the environmental impact of an introduced agent – data requirements and methodologies

3.1. *Establishment*

The potential of an exotic natural enemy to establish will influence its impact on non-target species, and may therefore determine the extent of other tests/information needed for the environmental risk assessment. The time scale of establishment can be categorised as follows:

1. no reproduction: one generation,
2. reproduction, but no survival during warm or cold season: one season, and
3. reproduction and long-term survival: multiple seasons.

The temporal scale also puts limits to the spatial scale of establishment. At the first and second time scale 'establishment' will be restricted to the area around the crop that can be reached by the animals released. Only when the agent can reproduce in non-target habitats are they able to affect larger areas (assuming dispersal abilities to be the same). Multiple-season introductions allow the agent to establish in and potentially affect even wider areas, as it has more time to spread.

The potential for establishment of the natural enemy should be concluded from the requirements of the natural enemy and opportunities offered in the area of release with respect to:

a. abiotic factors – does the climate between area of origin and area of release match?
b. biotic factors – availability of non-target species suitable for reproduction, temporal and/or spatial matching of non-target hosts and biological control agent, opportunities for winter survival? and
c. combined biotic and abiotic factors – are other resources, like refuges, for survival and reproduction available?

Data from the literature may be sufficient to determine the chances of establishment, but it may also be necessary to carry out laboratory and semi-field tests to prove whether establishment is possible or not in the target area or in surrounding non-target areas. If information indicates a very low probability that an agent can establish, the environmental assessment that follows may be less extensive than in case of high potential for establishment. Further discussion on factors relating to establishment potential of non-native arthropods can be found in Bale and Walters (2001).

3.2. *Dispersal*

It is important to determine the potential for dispersal of the biological control agent in order to evaluate the probability of temporal and spatial encounter between the biological control agent and non-target species. The risk of encounter is based on the mechanism of dispersal, life-span of the organism, and the local climate and habitat conditions in the area of release. We propose that if the agent does not disperse actively or passively for more than 10 meters per season (e.g. soil inhabiting entomopathogenic nematodes and fungi), it is likely that no further information or studies are needed. If the agent does not establish, but does disperse, the most relevant dispersal experiments can be done in the target area. The starting point for these is to make an inventory of non-target species over time, space and habitat. Transect studies may then be performed to measure dispersal speed (distribution of distances over time) and the numbers of individuals dispersing (relative to numbers present) under normal climate and habitat conditions (in this case the dispersal behaviour process is measured; Dingle, 1996; see for *Trichogramma*: Babendreier et al., in prep; see for whitefly parasitoids: Bellamy and Byrne, 2001; Loomans et al., in prep.). An alternative approach could be to count the number of hosts attacked, instead of the number of natural enemies dispersed (in this case the outcome of the dispersal process is tested; Dingle, 1996). Attack of non-target hosts in various habitats should be checked, but also target insect on target host plant should be offered in these habitats. In this way presence of a biological control agent can be observed, and conclusions be made concerning the dispersal potential, and also whether any non-targets are actually utilised in the non-target areas. If the agent can establish then determining dispersal in the novel environment is not possible, but similar experiments can be done in country of origin to estimate dispersal capabilities. In addition, any information on the possibility for secondary dispersal (e.g. mechanical, with crop or with vectors) should be provided.

3.3. *Host range*

Testing for host specificity of a biological control agent, or, in other words, determining the host range, is extensively described, as (1) this will form the focal point of environmental risk assessment, and (2) very limited information has been published about host-range testing procedures and protocols. If a natural enemy is very specific – attacking only one (monophagous) or a few related (oligophagous) hosts, then determination of direct and indirect effects on non-target species can be limited. Also, establishment and dispersal are not considered negative if the natural enemy is host specific. In this section the word host is considered synonymous with prey. Below, we attempt to design a testing scheme for host specificity of natural enemies. Because of the large variation in natural enemy – host relationships, the testing sequence should be considered as a basic approach, which will need to be adapted for specific situations. Before a specific testing scheme is designed, the following points need to be thought over.

1. The rearing of the host plant, host and natural enemy species previous to testing should be described in a detailed way, among others to be able to trace effects of conditioning and learning. Learning behaviour by a natural enemy and the presence of semiochemicals from the host or host plant or their interaction, may influence host acceptance patterns (Vet and Dicke, 1992; Vet et al., 1995).
2. Test conditions should be described in a detailed way, as they may strongly influence host acceptance, for example as a result of conditioning and learning. Further, the host plant and host used in testing should be specified.
3. During testing, the target and non-target hosts should be offered in a natural host distribution pattern, on the natural host plant or part of that or on an alternative host plant, which is not repellent for natural enemy (van Dijken et al., 1986; Follett et al., 2000; Sands, 1988).

The choice of non-target species is difficult but critical. A procedure similar to the phylogenetic centrifugal method used for evaluation of weed biological control agents is proposed, because it has been proved successful in selection of phytophagous insects for control of weeds and has a sound scientific basis (Wapshere, 1974; Lonsdale et al., 2001). This procedure is starting with testing non-target host species from the same genus, then progressing to those from the same tribe, subfamily, etc. If none of the non-target species from the same genus is attacked, one can stop testing non-targets that are related to the target. If several species within the same genus as the target are attacked, then it would be appropriate to test non-targets from the same tribe, and so on. Depending on the breadth of the host range of the natural enemy, several categories of other non-target species may need be tested, such as (a) non-related non-targets that occur in the same habitat of the target and are prone to attack, (b) non-related non-targets that occur in other habitats that are explored by the natural enemy, and (c) certain non-related threatened, economic, or aesthetic (symbolic) species. Available knowledge about host spectrum and habitats that are explored by natural enemy can help in narrowing down the non-targets to be tested. Generally, native hosts that are phylogenetically related to the original host of a newly introduced natural enemy may be attacked, and accidentally imported pests may be attacked by a native natural enemy of a related native phytophagous species (e.g. Viggiani and Gerling, 1994). In a number of cases, host-specificity data from mono- or slightly oligophagous species found in the literature were confirmed when exposed to new non-target host species (e.g. Cameron and Walker, 1997). In contrast, other natural enemy species that were considered to be monophagous or that had a rather restricted host range, were found to attack a number of other host species in the area of release (e.g. Brower, 1991; Barratt et al., 1997). Perhaps even more surprising was the finding that a natural enemy shown to be polyphagous at one place, appeared to perform as a monophagous natural enemy after introduction in another region (Salerno, 2002). Conclusions about host specificity can, therefore, seldom be made alone on data collected in the area of origin of the biological control agent, although Kuhlmann et al. (2000) suggest that this is an important first step.

Special consideration should be given in designing tests for host range of polyphagous predators where host size and location might be a better guideline than phylogenetic relatedness. Moreover, a wider host range needs to be tested than with many parasitoids because more intraguild predation is expected, as well as higher up trophic level effects. Specific (micro-) habitat demands of the predator may limit its degree of polyphagy and may make it less risky. Other categories needing care with testing are generalist parasitoids and (facultative) hyperparasitoids.

Within the EU-ERBIC project a sequential test for determining host ranges of natural enemies was developed, which is described in Table 1 and summarised in Figure 1. The test sequence may be changed if this can be motivated based on the biology of the natural enemy (e.g. Babendreier et al., 2002a, b). Step 1 and 2 can often generally be combined.

3.4. *Direct effects of released organism on other organisms in ecosystem*

3.4.1. *Effects on non-target herbivores*

The released biological control agent might affect the abundance of native non-target species in natural or semi-natural ecosystems, but earlier charges that natural enemy introductions have led to a strong reduction or even extinction of non-target species (Howarth, 1985, 1991, 2000), have later been said

Table 1. Sequential test to determine the host range of a natural enemy

Step 1: Petri dish non-choice black box test

The aim of the test is to answer the question: does the biological control agent attack the non-target organism in the appropriate stage? A positive control is performed with the target species; a negative control is done with the target species *without* the natural enemy to check survival etc. of target species under test conditions. The non-target species are selected according (1) to their phylogenetic relationship with the target, (2) occurrence in the same microhabitat and prone to attack, and (3) their status as endangered species (Lonsdale et al., 2000). If none of the non-targets is attacked and the pest (control) species is attacked, one can stop testing, and no direct effects on non-target species in field are expected. If non-target species were attacked, go to step 2. Long-term behavioural observations are not done in step 1, but it is suggested to check the activity (searching or not) of the natural enemy at the start of testing, and after an interval of about 30 minutes to be sure that lack of attack in tests is not the effect of poor condition of natural enemies, but of rejection of the non-target.

Step 2: Petri dish non-choice behavioural test

The aim of the test is to answer the question: does the biological control agent attack the non-target organism consistently? A positive control is performed with the target species; a negative control is done with the target species *without* the natural enemy. Check encounter and attack rate over time for non-target species to determine possible increase in acceptance due to increasing oviposition/predation pressure. If non-target is not attacked at all and the pest (control) species is attacked, one can stop testing for that species, and no direct effects on that non-target species in field is expected. If non-target is only attacked at the end of the observation period, then the risk of direct effects on that species is relatively small. If non-target host is attacked for a constant percentage, then the risk might be considerable. For non-target species that are attacked, go to step 3. This non-choice test can also be done with sequential alternate exposure of target and non-target to avoid risk of oviposition in non-target, and thus to avoid inclusion of false positives in the list of non-target species. Observations can, when behaviour of natural enemy is known, be automated.

Step 3: Petri dish choice test

The aim of the test is to answer the question: does the biological control agent attack the non-target when the target species is present? Choice test with target and non-target host. A positive control is performed with the target species; a negative control is done with the target species *without* the natural enemy. Check encounter and attack rate over time for non-target and target, to determine host preference, eventual shifts in preference and a possible increasing attack pressure of usually not attacked hosts, because the preferred host is no longer available. No or low attack of non-target and no shift in host preference over time: low risk for direct effects on non-target. If non-target is attacked in choice test, but not in no-choice test, this may be a spill-over effect ('confusion' of natural enemy), and non-target is likely to be outside host range, but do check whether it can develop on the attacked non-target). If non-target is easily attacked either from start onwards, or later during the observation, go to step 4. Observations can, when behaviour of natural enemy is known, be automated.

Table 1. Continued

Step 4: Large cage choice test

The aim of the test is to answer the question: does the biological control agent attack the non-target when the target species is present in a semi-natural situation? Present multiple host plants with various non-target and target hosts to biological control agent in a large cage. Offer target and non-target hosts in as natural a situation as possible and on their natural host plants. A positive control is done in the same type of cage with only target host; a negative control is done with the target species, but without the natural enemy. Determine encounter and attack rates over time. For interpretation of results, see step 3. Non-target species that are easily attacked on their host plants pose a high risk for non-target effects.

Step 5: Field test

The aim of the test is to answer the question: does the biological control agent attack the non-target when the target species is present in a natural situation? This test can only safely be done if biological control agent cannot establish in target area (e.g. agents from tropical areas to be used in greenhouses in temperate climates)! Release natural enemy in non-target habitat, determine attack of non-target species. Control: put target species on target host plant in the non-target habitat. If target species is easily attacked, and no or low attack of non-target occurs, this indicates a low risk for direct effects on non-target. Non-target species that are easily attacked on their host plants in their habitat pose a very high risk for non-target effects.

to be unjustified (e.g. Funasaki et al., 1988; Follett et al., 2000). Direct negative effects can occur when the agent attacks other species in addition to the target species (Figure 2). Knowledge on host specificity is therefore essential for any inquiry in potential direct effects.

However, even when the biological control agent attacks a non-target species, it does not have to affect its abundance. Attack rates or population densities under field conditions may be relatively low. The non-target population may already be limited by other factors (such as resource availability or other natural enemies) that will be relaxed when attack rates increase, and (partly or fully) compensate the impact of the biological control agent.

Moreover, even when biological control agent somehow affects the abundance of a non-target organism it is unlikely that it will lead to (local) extinction. In nature, it is the rule rather than the exception to find extremely low densities of both herbivores and their natural enemies, and these natural enemies are a substantial component of biodiversity. The search behaviour of natural enemies generally leads to the decision to leave host patches before parasitising or eating all hosts or prey. Further, hosts have mechanisms of escaping their natural enemies in space and time, which reduces the chances of the host from going extinct. Finally, asynchrony between local dynamics allow for large-scale persistence even when local extinctions occur (meta-

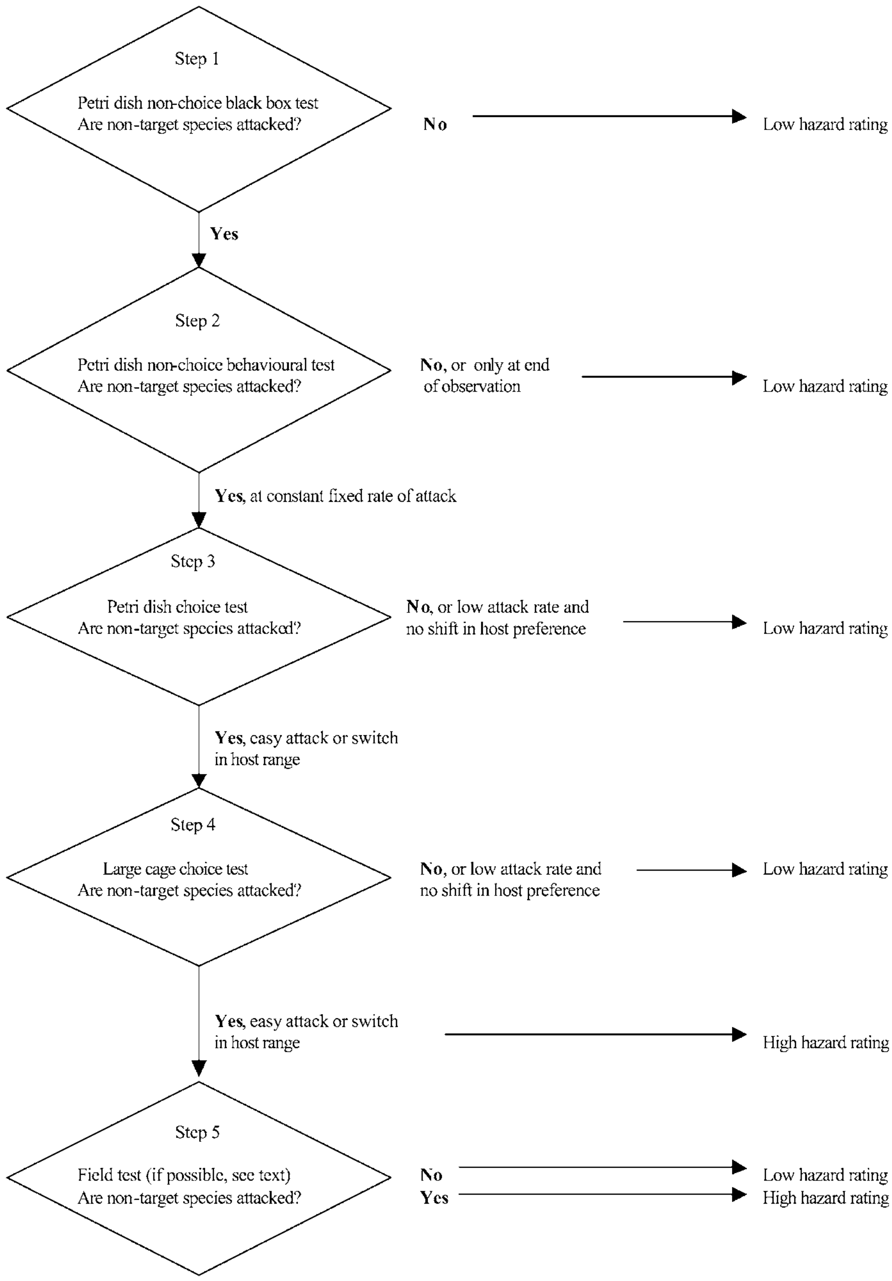


Figure 1. Flow chart summarising host-specificity testing.

population dynamics, Hanski and Singer, 2001). Pests have, consequently, seldom if ever been exterminated in the more than 100 years of insect biological control. Rather, a low population level of both pest and natural enemy developed, like in natural ecosystems.

3.4.2. *Effects on other trophic levels: Intraguild predation and omnivory*
When the released biological control agent is able to attack not only herbivores but also species that are feeding on these herbivores themselves ('intraguild predation' (Rosenheim et al., 1995) or 'facultative hyperparasitism' (Sullivan, 1987)) this is of specific interest, as these other natural enemies might be important in the regulation of some of these herbivores (see indirect effects).

Some natural enemies also feed on plant material during part of their life cycle (omnivory, Coll and Guerson, 2002). Information on the effect on plants by these agents should be provided.

3.4.3. *Enrichment and vectoring*
The released biological control agent may not only attack some organisms but may also be attacked by other organisms within the ecosystem. This will have positive rather than negative effects on these populations, but it may indirectly have negative impact on victim species (see indirect effects). A special case is when the natural enemy can act as vector for pathogens (Bjørnson and Schütte, 2003). Information on potential pathogen transfer by these agents should be provided.

For the environmental risk analysis, any known or potential direct effects should be reported. If intraguild predation or hyperparasitism are indicated in the literature for related natural enemies, or can be expected from their biology, then intraguild predation/hyperparasitism should be investigated case by case (as part of the host-range testing). Based on its ability to establish, its attack rate on the non-target species, and the regulatory mechanisms present in the non-target population, potential effects of the biological control agent can be estimated. When a serious impact is possible, further testing in the area of origin may be required. Finally, a conclusion concerning risk should be drawn.

3.5. *Indirect effects of released organism*

Depending on the trophic position of the directly affected species in the ecosystem the following indirect effects can be expected (see also Figure 2).

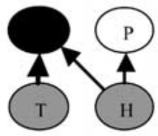
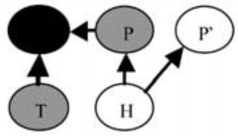
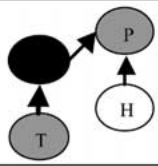
Direct effects	→	Indirect effects
1. Attack of non-target herbivore (H)		Competition (for H) with other natural enemy (P)
2. Attack of other natural enemy, P (Intraguild Predation)		- Enemy escape of herbivore (H) - Competitive release of 3rd natural enemy (P')
3. Enrichment or vectoring of other (top or intraguild) natural enemy or pathogen (P)		Apparent competition or pathogen transfer between agent and non-target host (H)

Figure 2. Possible direct effect of the release of a biological control agent on native populations and some of its indirect effects, based on trophic interactions. In diagram: arrows indicate flow of energy; circles indicate populations (black for the agent, grey for directly affected, white for indirectly affected populations; T = target).

3.5.1. *Competition*

When the biological control agent is attacking and reducing a (target or non-target) herbivore population this may negatively affect other natural enemies that feed on this resource. When the biological control agent and the other natural enemy are effectively exploring the same resource ultimately only one of both may survive (competitive exclusion, Tilman, 1982). For example, it has been suggested that in some cases releases of polyphagous predators has not only led to a decimation of pest caterpillars, but also to a reduction of non-target caterpillars resulting in a decline in native predaceous wasp and bird populations (Simberloff, 1992).

3.5.2. *Indirect effects of intraguild predation*

When the agent is an intraguild predator (or hyperparasitoid) of another natural enemy (see 3.4.2) its suppression may indirectly reduce the predation on its (usually herbivorous) prey population (Rosenheim et al., 1995; Brodeur and Rosenheim, 2001). This may lead to temporal outbreaks, or ultimately to the increase of those carnivores that that have been released from its competitors (Polis and Holt, 1992).

3.5.3. *Effects of enrichment (apparent competition)*
When the agent is an (additional) food source for another natural enemy (an intraguild predator or a top predator), its introduction may temporarily release or increase the predation pressure on the carnivore's prey, depending on the behavioural response of the natural enemy (Holt and Kotler, 1987). On a somewhat longer time scale it is expected to result in an increase of the natural enemy population. Ultimately this may result in a decrease of its prey population (apparent competition between the agent and the other prey, Holt, 1977). It is, however, unlikely that the released biological control agents contribute significantly to the resource pool of any species in the ecosystem, unless it is a relatively specialized (hyper)parasitoid.

3.5.4. *Other indirect effects*
Any of the indirect effects may in its turn result in other indirect effects in the ecosystem. Apart from effects resulting from linked trophic interactions, also other indirect effects can occur by effecting non-food requirements of other species, such as protection, pollination and (seed) dispersal.

3.5.5. *Hybridization*
Apart from causing ecological changes, a released biological control agent may also cause genetic changes in other populations in the ecosystem. One specific mechanism is hybridisation between the biological control agent and indigenous biotypes of same or very closely related natural enemy species, which need specific attention in the risk analysis.

For the environmental risk analysis, any known indirect effects or potential indirect effects on individual species and/or ecosystem should be reported. Indirect effects via target organisms (e.g. lower numbers of native natural enemies as a result of reduction of target pest) are generally accepted, and not considered negative. But indirect effects via non-target organisms on population and community level are usually considered negative. The problem is that each direct effect on a non-target is expected to result in a multitude of (small to large) indirect effects, and these can be positive, neutral or negative. Existing information on these effects is very limited, and estimating indirect effects is difficult. If the exotic biological control agent is expected to attack non-target species in high numbers, the direct and indirect effects will generally be considered too serious.

4. Proposed risk assessment methodology for natural enemies

Evaluation of risks related to releases of natural enemies demands integration of many aspects of their biology, as well as information on ecological interactions identified above. For a full risk assessment, three steps are distinguished: (1) the risk identification and evaluation procedure concerning the release of a natural enemy, (2) a risk management plan dealing with risk reduction and risk mitigation, and (3) a risk/benefit analysis of the proposed release of the natural enemy, together with risk/benefit analyses of current and alternative pest management methods.

4.1. *Risk identification and evaluation*

Normally, for a risk evaluation, one will identify the hazards, and determine the probabilities that hazards will materialise. The hazard of a biological control agent can be defined as any imaginable adverse effect, which can be named and measured, such as direct and indirect adverse effects on non-target organisms and adverse effects on the environment. The risk of adverse effects of the release of a biological control agent is the product of the impact of likelihood (probability) and the impact of magnitude (consequence). Here the system proposed by Hickson et al. (2000) for environmental risk management in New Zealand is used as a starting point for development of a risk evaluation for biological control agents. The Hickson et al. paper is based upon the risk management system proposed by the Australian/New Zealand Standard 4360: 1999 Risk Management. In this system, five groups of risks are considered related to the release of exotic biological control agents: establishment, dispersal, host specificity, direct effects, and indirect non-target effects.

In order to assess risks, first the likelihood (Table 2a) and the magnitude (Table 2b) of adverse effects are estimated and are then placed in a matrix according to the approach of Hickson et al. (2000) (Table 2c), where magnitude has a greater weight than likelihood. As many of the descriptions given by Hickson et al. (2000) could not be used to estimate effect for the five groups of risks given above for biological control agents, a new list of descriptions for likelihood and magnitude are proposed in Tables 3 and 4. The new descriptions for likelihood and magnitude given in Tables 3 and 4 are a first attempt for qualification and quantification of risks posed by natural enemies, and are based on experience gained in the ERBIC project. The criteria will depend on the type of hazard and the aim of release (inundative or classical biological control) and will therefore need adaptation for specific cases. For example, should all criteria be valued equally or should a weighting factor be applied under certain conditions (e.g. does establishment and dispersal over large distances matter if the natural enemy only attacks a few non-target species in very low numbers)? Or, let us suppose that a pristine, rare and small size non-target habitat will be invaded by the natural enemy, and serious direct and indirect non-target effects might be the result.

Table 2. Qualitative scales for likelihood (a), magnitude (b) and level of risk of adverse effects (c) (after Hickson et al., 2000)

(a) Likelihood	**Description**
Very unlikely	Not impossible but only occurring in exceptional circumstances
Unlikely	Could occur but is not expected to occur under normal conditions
Possible	Equally likely or unlikely
Likely	Will probably occur at some time
Very likely	Is expected to occur

(b) Magnitude	**Description**
Minimal	Insignificant (repairable or reversible) environmental impact
Minor	Reversible environmental impact
Moderate	Slight effect on native species
Major	Irreversible environmental effects but no species loss, remedial action available
Massive	Extensive irreversible environmental effects

(c) Level of risk of adverse effect

	Magnitude				
Likelihood	Minimal	Minor	Moderate	Major	Massive
Very unlikely	Insignificant	Insignificant	Low	Medium	Medium
Unlikely	Insignificant	Low	Low	Medium	High
Possible	Low	Low	Medium	Medium	High
Likely	Low	Low	Medium	High	High
Very likely	Medium	Medium	High	High	High

In this case the classification of Table 4 is no longer relevant and the decision will be not to import and release this natural enemy. After the risk evaluation procedure, biological control experts with knowledge of the natural enemy under consideration give an expert judgement and conclude either that the release of the natural enemy is potentially acceptable or unacceptable, or that more information is needed.

4.2. *Risk management*

The next step of the risk assessment process is to discuss risk management, including risk mitigation and risk reduction. If an exotic biological control agent is expected to cause significant adverse effects on non-target organisms a permit for releases will not be issued. If the exotic organism itself is

Table 3. Proposed new descriptions of likelihood for establishment, dispersal, host range, direct and indirect effect (*as in Hickson et al., 2000)

Establishment[1]* in non-target habitat	Dispersal[2] potential	Host range[3]	Direct* and indirect* effects
Very unlikely	< 10 m	0 species	Very unlikely
Unlikely	< 100 m	1–3 species	Unlikely
Possible	< 1,000 m	4–10 species	Possible
Likely	< 10,000 m	11–30 species	Likely
Very likely	> 10,000 m	> 30 species	Very likely

[1]The propensity to overcome adverse conditions (winter or summer: physical requirements) and availability of refuges.
[2]Distance moved per release (take number of generations per season into account); determine dispersal curve, sampling points at 10, 100 and 1000 m, sampling period is 50% life span.
[3]The propensity to realise its ecological host range in the release area.

Table 4. Proposed new descriptions of magnitude for establishment, dispersal, host range, direct and indirect effects

Magnitude	Establishment[1] in non-target habitat	Dispersal[2] potential	Host range[3]	Direct[4] and indirect[5] effects
Minimal	local (transient in time and space)	< 1%	species	< 5% mortality
Minor	< 10%	< 5%	genus	< 40% mortality
Moderate	10–25%	< 10%	family	> 40% mortality and/or > 10% short term population suppression
Major	25–50%	< 25%	order	> 40% short term population suppression, or > 10% permanent population suppression
Massive	> 50%	> 25%	none	> 40% long term population suppression or local extinction

[1]Percentage of potential non-target habitat where biological control agent may establish.
[2]Percentage of released biological control agent dispersing from target release area.
[3]Taxon range that biological control agent attacks.
[4]Direct effect: mortality, population suppression or local extinction of directly affected non-target organisms; see Lynch et al. (2001) for details.
[5]Indirect effect: mortality, population suppression or local extinction of one or more species of non-target species that are indirectly influenced by the released biological control agent.

considered safe, but the host-plant and host on which it is shipped might pose risk, procedures may be imposed to prevent problems. Such procedures may consist of directions for shipment and materials used, screening and destruction of contaminants after arrival in country of release (e.g. check for pathogens and biotype of host insect, and rearing of biological control agent under quarantine for one or more generations in order to clean the population from hyperparasitoids or entomopathogenic microorganisms). For an example of risk management issues, we refer to Cross and Noyes (1995). In some cases, risks may be minimised by imposing label restrictions concerning for example the types of crops on which the use of the organism is or is not allowed (e.g., treatment of flowering plants with a mycoinsecticide), or by requesting specific application techniques (e.g., soil incorporation only for insect pathogenic nematodes).

4.3. *Risk/benefit analysis*

The final step in making a justified environmental risk analysis for a new biological control agent, is to conduct a risk benefit analysis which should include a comparative performance of pest management methods, particularly based on environmental aspects. The environmental benefits of use of the proposed biological control agent should be compared to environmental effects of currently used and other alternative control methods. To be able to make a comparative performance analysis, information as specified below should be available for all control methods: (1) The pest control level that can be obtained, (2) The total cost of applying a pest control method to reach a sufficient level of control (labour, equipment, control agent/pesticide, etc.), (3) The costs to correct for development of resistance, (4) The amount of positive effects on environment (effect on biodiversity; reduction of environmental pollution) (5) The amount of negative effects on environment (negative effects on biodiversity, such as non-target effects, negative effects on pollinators, fish and wildlife, and negative effects on native natural enemies resulting in a reduction of natural pest control; contamination of soil, water and air; costs to correct for these negative effects), and (6) The effects on human health. When data are not available, expert judgement may suffice for some of these items.

5. Application of proposed risk assessment methodology to currently used biological control agents

In this section the proposed risk assessment methodology is applied to a number of natural enemies species – mainly exotic, but also native – which

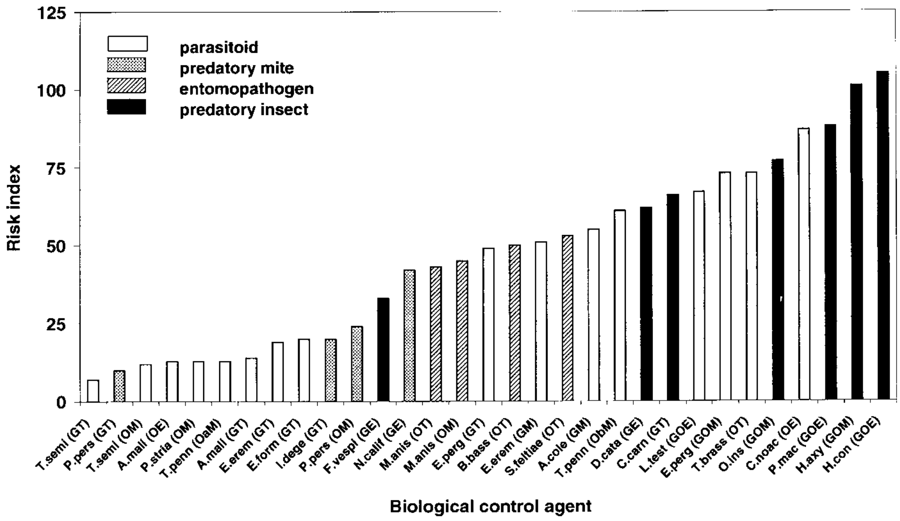


Figure 3. Risk indices of commercially available inundative and classical biological control agents released in European and Mediterranean countries. Names of organisms: first letter of genus name, three or more letters from species name. For full names see Table 5. Letters added to name of organisms: a = risk index after release, b = risk index before release, G = greenhouse, O = open field, M = Mediterranean climate, T = Temperate climate, E = across Europe.

are currently mass-released for biological control of pests in greenhouses and open field. Species were chosen from the ERBIC case studies, and from the EPPO 'list of biological control agents widely used in the EPPO region' (EPPO Standard PM 6/3, 2002), which is in preparation. Further, we assessed several typical classical and inundative biological control agents, and some species that would be classified as 'doubtful' by biological control specialists.

Tables 3 and 4 give criteria to evaluate the potential risks of release of a natural enemy. However, without adding a numerical value to each criterion it remains a qualitative procedure, making comparison of natural enemies difficult. Therefore, we first gave the following values to each criterion: Likelihood: very unlikely = 1, unlikely = 2, possible = 3, likely = 4, very likely = 5; Magnitude: minimal = 1, minor = 2, moderate = 3, major = 4, massive = 5.

Having done that, we have applied the criteria to calculate a risk index to the natural enemies (Figure 3 and Table 5). The overall risk index for each natural enemy is obtained by first multiplying the figures obtained for likelihood and magnitude, and then by adding the resulting figures obtained for dispersal, establishment, host specificity, direct and indirect effects without weighing. The maximum score is 125 ($5 \times 5 \times 5$).

In this paper we present the results of the evaluations without discussing in detail how we assigned values to each criterion and without providing all references containing data that we used. This information is presented on the website of the ERBIC project (http://honeybee.helsinki.fi/MMSBL/MAEL/Hankkeet/ERBIC/index.htm) and in the final report of the ERBIC project (Hokkanen et al., 2002).

Now, we have come to the difficult task of classifying biological control agents as safe, risky or intermediate. As a premise, we want to state here that our approach is but a first attempt to quantify risk, with the aim to open a discussion that may lead to better ways of pre-introductory evaluation of the risk of release of biological control agents. We also want to state explicitly that interpretation of risk indices should be done with great care, and can only be done by biological control experts knowing the biology of the natural enemy under consideration. Further, it should be clearly stated for which region a particular risk assessment was made (continent, part of continent, ecoarea, country, part of country, etc), because risk indices will vary according to the region for which they were made. It would be best to determine indices for ecoareas, because these are rather well defined and relevant biological units (ecoarea: an area with similar fauna, flora and climate and hence similar concerns about the introduction of biological control agents; FAO, 1999). However, risk of dispersal from an ecoarea to nearby areas with suitable climates should be considered in the risk assessment. We have used a pragmatic approach in this paper and assigned natural enemies to countries or the European continent. Finally, risk indices should not be seen as absolute values, but as indicators to which a judgement can be connected for granting permission to release or not. We propose to use them within certain risk categories (low, intermediate, high risk). Based on our collective experience in biological control, and after having discussed the risk indices obtained with our rating system, we propose to use the following risk index categories: risk indices lower than 35 points will generally result in a proposal of no objection against release of the agent, a risk index higher than 70 points will generally result in the advise not to release the agent, and intermediate risk indices between 35 and 70 points will result in the advise to come up with additional information before a conclusion concerning release will be drawn.

Dealing with uncertainty is a common aspect of risk assessments. In cases where data for a certain characteristic are not available, or when insufficient or unclear data are provided, the maximum value for likelihood and/or magnitude is assigned to that characteristic in order to take uncertainty into account. When this leads to a classification of intermediate or high risk, the applicant will be asked to provide new data. In these cases, the evaluating

Table 5. The risk assessment methodology applied to some commercially available inundative and to some classical biological control agents released in European and Mediterranean countries

Biological control agent (origin)	Area of release (use in)		Establish-ment	Dispersal	Host range	Direct effects	Indirect effects	Risk index (sum L × M's)	Type of agent[1]	Reference
Aphelinus mali	Europe	Likelihood	5	5	1	1	1		par	Greathead, 1976
(eastern Nearctic)	(open field)	Magnitude	1	1	1	1	1			Mols and de Boer, 2001
classical biocontrol; 70 years	after release	L × M:	5	5	1	1	1	13		Cross et al., 1999
Aphidius colemani	Italy	Likelihood	4	3	4	4	3		par	Rabasse and van Steenis, 1999
(Palearctic, Mediterranean)	(greenhouse)	Magnitude	3	3	4	3	2			
inundative biocontrol		L × M:	12	9	16	12	6	55		
Aphytis melinus	Belgium	Likelihood	1	2	3	1	1		par	Rosen, 1994
(India/Pakistan)	(greenhouse)	Magnitude	1	1	3	1	1			
inundative biocontrol		L × M:	1	2	9	1	1	14		
Beauvaria bassiana	Finland	Likelihood	2	2	5	5	3		entomop	Vestergaard et al., 2002
(Cosmopolitan, Finland)	(open field)	Magnitude	1	1	5	3	2			Vänninen et al., 2000
inundative biocontrol		L × M:	2	2	25	15	6	50		ERBIC case study
Cales noacki	Europe	Likelihood	5	5	3	5	3		par	Viggiani, 1994
(Chile)	(open field)	Magnitude	5	5	3	5	1			
classical biocontrol; 20 years	after release	L × M:	25	25	9	25	3	87		
Chrysoperla carnea	Switzerland	Likelihood	5	4	5	5	3		predins	Canard et al., 1984
(Cosmopolitan)	(greenhouse)	Magnitude	5	2	5	1	1			
inundative biocontrol		L × M:	25	8	25	5	3	66		
Delphastus catalina	Europe	Likelihood	5	4	5	5	5		predins	Liu and Stansly, 1999
(Nearctic/Neotropic)	(greenhouse)	Magnitude	4	3	3	2	1			Heinz et al., 1999
inundative biocontrol		L × M:	20	12	15	10	5	62		

Encarsia formosa	Netherlands	Likelihood	1	3	2	5	5		par	ERBIC case study
(southern Nearctic)	(greenhouse)	Magnitude	1	1	3	1	1			
inundative biocontrol		L × M:	1	3	6	5	5	20		
Encarsia pergandiella	Italy	Likelihood	5	3	4	5	5		par	Viggiani and Gerling, 1994
(southern Nearctic)	(greenhouse/	Magnitude	5	1	5	4	1			Briggs and Collier, 2001
classical and inundative	open field)	L × M:	25	3	20	20	5	73		ERBIC case study
Encarsia pergandiella	Netherlands	Likelihood	1	3	4	5	5		par	ERBIC case study
(southern nearctic)	(greenhouse)	Magnitude	1	1	5	4	1			
If applied as inundative biocontrol		L × M:	1	3	20	20	5	49		
Eretmocerus eremicus	South Europe	Likelihood	5	4	2	4	4		par	ERBIC case study
(southern Nearctic)	(greenhouse)	Magnitude	5	2	3	2	1			Bellamy and Byrne, 2001
inundative biocontrol		L × M:	25	8	6	8	4	51		
Eretmocerus eremicus	Netherlands	Likelihood	1	4	2	4	4		par	ERBIC case study
(southern Nearctic)	(greenhouse)	Magnitude	1	1	3	1	1			Bellamy and Byrne, 2001
If applied as inundative biocontrol		L × M:	1	4	6	4	4	19		
Franklinothrips vespiformis	Europe	Likelihood	1	2	4	5	5		predins	Loomans and Vierbergen, 1999
(Asia)	(greenhouse)	Magnitude	1	1	5	1	1			Hoddle et al., 2000
inundative biocontrol		L × M:	1	2	20	5	5	33		
Harmonia axyridis	Italy	Likelihood	5	4	5	5	5		predins	Burgio et al., 2002
(eastern Palearctic)	(greenhouse/	Magnitude	4	4	5	4	4			Tedders and Schaefer, 1994
inundative biocontrol	open field)	L × M:	20	16	25	20	20	101		ERBIC case study
Hippodamia convergens	Europe	Likelihood	4	4	5	5	5		predins	Colfer and Rosenheim, 2001
(western Nearctic)	(greenhouse/	Magnitude	5	5	5	4	4			Obrycki and Kring, 1998
inundative biocontrol	open field)	L × M:	20	20	25	20	20	105		
Iphiseius degenerans	Netherlands	Likelihood	1	1	4	2	2		predmite	van Rijn and Tanigoshi, 1999
(Africa/Mediterranean)	(greenhouse)	Magnitude	1	1	2	3	2			
inundative biocontrol		L × M:	1	1	8	6	4	20		

Table 5. Continued

Biological control agent (origin)	Area of release (use in)		Establish-ment	Dispersal	Host range	Direct effects	Indirect effects	Risk index (sum L × M's)	Type of agent[1]	Reference
Lysiphlebus testaceipes	Europe	Likelihood	5	3	5	5	5		par	Nicoli and Burgio, 1997
(Nearctic, Cuba)	(greenhouse/	Magnitude	4	4	3	3	1			Stáry et al., 1988a, b
inundative biocontrol	open field	L × M:	20	12	15	15	5	67		
Metarhizium anisopliae	Finland	Likelihood	3	1	5	1	1		entomop	Husberg and Hokkanen, 2000
(Cosmopolitan, Finland)	(open field)	Magnitude	5	1	5	1	1			Vänninen et al., 2000
inundative biocontrol		L × M:	15	1	25	1	1	43		ERBIC case study
Metarhizium anisopliae	Spain	Likelihood	4	4	3	4	2		entomop	Lomer et al., 2001
var. *acridum* (Cosmopol.,	(open field)	Magnitude	2	2	5	3	1			ERBIC case study
Sahelian Africa)		L × M:	8	8	15	12	2	45		
inundative biocontrol										
Neoseiulus californicus	Europe	Likelihood	3	1	3	5	5		predmite	Croft et al., 1998
(southern Nearctic)	(greenhouse)	Magnitude	2	1	5	2	2			
inundative biocontrol		L × M:	6	1	15	10	10	42		
Orius insidiosus	Italy	Likelihood	5	4	5	5	5		predins	Tommasini et al., 2002
(eastern Nearctic)	(greenhouse/	Magnitude	5	3	5	2	1			Alvarado et al., 1997
inundative biocontrol	open field)	L × M:	25	12	25	10	5	77		ERBIC case study
Phytoseiulus persimilis	Italy	Likelihood	5	1	2	2	1		predmite	Helle and Sabelis, 1985
(Mediterranean)	(open field)	Magnitude	3	2	2	1	1			
inundative biocontrol		L × M:	15	2	4	2	1	24		
Phytoseiulus persimilis	Netherlands	Likelihood	1	1	2	2	1		predmite	Helle and Sabelis, 1985
(Mediterranean)	(greenhouse)	Magnitude	1	2	2	1	1			
inundative biocontrol		L × M:	1	2	4	2	1	10		

Podisus maculiventris	Europe	Likelihood	4	4	5	5	5		predins	DeClerq, 2000
(Nearctic/Neotropic)	(greenhouse/	Magnitude	4	3	5	5	2			
inundative biocontrol	open field)	L × M:	16	12	25	25	10	88		
Polynema striaticorne	Italy	Likelihood	5	5	1	1	1		par	Alma et al., 1987
(Nearctic)	(open field)	Magnitude	1	1	1	1	1			
classical biocontrol; 30 years after release		L × M:	5	5	1	1	1	13		
Steinernema feltiae	Finland	Likelihood	3	1	5	4	4		entomop	Ehlers and Hokkanen, 1996
(Holarctic)	(open field)	Magnitude	5	1	5	2	1			ERBIC case study
inundative biocontrol		L × M:	15	1	25	8	4	53		
Thripobius semiluteus	South Europe	Likelihood	4	4	2	1	1		par	Viggiani et al., 2000
(Subtropical, Tropical)	(open field)	Magnitude	1	1	1	1	1			
inundative biocontrol		L × M:	4	4	2	1	1	12		
Thripobius semiluteus	North Europe	Likelihood	1	3	1	1	1		par	Froud and Stevens, 1998
(Subtropical, Tropical)	(greenhouse)	Magnitude	1	1	1	1	1			
inundative biocontrol		L × M:	1	3	1	1	1	7		
Trichogramma brassicae	Switzerland	Likelihood	5	2	5	5	5		par	Babendreier et al., 2002
(Europe)	(open field)	Magnitude	5	4	5	2	1			Suverkropp, 1997
inundative biocontrol		L × M:	25	8	25	10	5	73		ERBIC case stdy
Trichopoda pennipes	Italy	Likelihood	5	5	4	5	5		par	Salerno et al., 2002
(Nearctic)	(open field)	Magnitude	3	2	4	2	2			
classical biocontrol; before release		L × M:	15	10	16	10	10	61		
Trichopoda pennipes	Italy	Likelihood	5	5	1	1	1		par	Salerno et al., 2002
(Nearctic)	(open field)	Magnitude	1	1	1	1	1			
classical biocontrol; 10 years after release		L × M:	5	5	1	1	1	13		

[1] par = parasitoid, predins = predatory insect, predmite = predatory mite, entomop = entomopathogen.

organisation will have to make clear what kind of additional information is needed.

Taking these considerations into account, we will draw several conclusions from the data presented in Table 5 and Figure 3.

- The lowest risk index found for an inundative biological control agent used in greenhouses is 7 (*Thripobius semiluteus*; no establishment, poor dispersal outside greenhouse, monophagous, no direct or indirect non-target effects) and 12 when used in the field (*Thripobius semiluteus;* some establishment, reasonable dispersal, monophagous, no direct or indirect non-target effects).
- The lowest risk index found for a classical biological control agent is 13 (e.g. *Aphelinus mali*, *Polynema striaticorne* and *Trichopoda pennipes*; good establishment and dispersal in target habitat but not in non-target habitats, monophagous, no direct or indirect non-target effects).
- Low risk indices (below 35) were found for many parasitoids, several predatory mites, and one predatory insect.
- Intermediate risk indices (between 35 and 70) were found for all guilds of natural enemies represented in Table 5: parasitoids, predatory insects, predatory mites, and parasitic nematodes and entomopathogenic fungi.
- Entomopathogens (*Beauveria, Metarhizium* and *Steinernema*) all score intermediate because of their broad host range, but their very limited dispersal capacities strongly reduces risk.
- The highest risk indices were found for predatory insects (*Harmonia axyridis* (101), *Hippodamia convergens* (105), *Podisus maculiventris* (88), *Orius insidiosus* (77)) and parasitoids (*Encarsia pergandiella* and *Trichogramma brassicae* (both 73), and *Cales noacki* (87)). This was not a surprise as they would all be classified by biological control experts in the high-risk category based on what is known of their biology.
- The risk assessment methodology as we applied it clearly results in different values for the same organism when evaluated for different release areas. The predatory mite *Phytoseiulus persimilis* has a higher risk index when released in the open field in Mediterranean Italy (24), than when released in greenhouses in temperate climate countries (10). The parasitoid *Encarsia pergandiella* has a higher risk index when released in greenhouse in Mediterranean Italy (72), than when released in greenhouses in temperate climate countries (49). The same holds for the following other species mentioned in Table 5: *Eretmocerus eremicus, Metharhizium anisopliae* and *Thripobius semiluteus*.
- Risk assessment methodologies may sometimes lead to underestimating risk, particularly if biological knowledge of the ecosystem where the natural enemy will be released is poor. For instance, when an agent

is reported monophagous in its area of origin and is subsequently released into a new region where the native host fauna has been poorly surveyed or examined, range expansion to non-target hosts might occur (e.g. Brower, 1991; Barratt et al., 1997). Prior to performing proper host specificity testing, adequate knowledge of the potential non-target species and habitats in the area of release is necessary. In Europe, where the arthropod fauna is relatively well known, this information is likely available. In other regions, however, where the native fauna has been poorly investigated, care should be taken with respect to whether the state of knowledge at pre-release and early post-release is sufficient to predict the occurrence of host range expansion and the magnitude of its ecological impact. The reconstruction of the introduction of the flower-head weevil, *Rhinocyllus conicus* as a weed biological control agent into North America, by Gassmann and Louda (2001) is a good example why things sometimes have gone wrong.

- Risk assessment methodologies may also sometimes lead to overestimating risk. Based on information from the literature, the tachinid parasitoid *Trichopoda pennipes* that was accidentally introduced in Italy more than 10 years ago would receive an intermediate risk index rating of 61. This rating is mainly the result of its reported polyphagy in its area of origin. After extensive sampling in the field in Italy 10 years since its introduction, it appeared to have established in central Italy, but it attacks only one host (the pest species *Nezara viridula*) and none of the other related native pentatomid species occurring in the same area (Salerno et al., 2002).

6. Discussion

Biological control with exotic natural enemies has been practised for more than a century. This activity has resulted in long-term, economic and environmentally benign solutions to severe pest, disease and weed problems. In contrast with chemical control, there is limited evidence that biological control of insects and mites has resulted in negative environmental or health effects. The current popularity of commercial inundative biological control may, however, result in problems, as an increasing number of activities will be executed by persons not trained in identification, evaluation and release of biological control agents. Therefore, a methodology for risk assessment has been developed and applied to a number of natural enemies currently used. With this methodology, meaningful ranking of natural enemies in risk categories appears possible.

Host specificity, as stated before, is the crucial element in the whole evaluation process, because lack of host specificity might lead to unacceptable risk if the agent establishes and disperses widely, whereas, in contrast, a monophagous biological control agent is not expected to create serious risk even when it establishes and disperses well. Compare the risk indices for host specific classical biological control agents (e.g. the parasitoids *Aphelinus mali* and *Polynema striaticorne*, both with a risk index of 13) with that of less host specific inundative biological control agent (e.g. *Aphidius colemani* (risk index of 55), and *Encarsia pergandiella* and *Trichogramma brassicae* (both with a risk index of 73)). We might have been rather conservative with our ranking of host range categories in Table 4. Based on evaluation of more natural enemies, we may have to decide to use the following charecterisation: minimal – single species (the target, so the natural enemy is monophagous), minor – one or two sibling species, moderate – genus, major – family, massive – order.

High values for establishment and dispersal are not by definition negative. When dealing with mono- or oligophagous natural enemies, establishment and dispersal to non-target habitats do not lead to increased risk. However, high values for establishment and dispersal are considered negative when the biological control agent has a wide host range. This illustrates that one cannot blindly use the numerical risk index values, and the evaluation process might be improved by applying weighting factors to the criteria, depending on possible hazard and on the type of biological control programme for which they are selected. Based on a further evaluation of a large number of currently used natural enemies, weighting factors maybe used for several criteria. For example, as indirect non-target effects are much more difficult to test for than direct effects, a greater uncertainty needs to be attached to it, and this could be done by adding a weighting factor to this criterion.

Extreme care should be taken with exotic agents that establish across the area of introduction, that spread actively by dispersal or migration, which have a broad host range and that have non-target habitats in common with related indigenous species, like for instance some coccinellid predators as *Harmonia* spp. Records from Palaearctic species introduced into North America, have shown range expansions over the past 30 years (Hoebeke and Wheeler, 1996; McCorquodale, 1998), resulting in non-target effects (Lynch and Thomas, 2000).

Some currently commercially available biological control agents obtain high-risk indices because of their wide host range and, thus, supposedly many non-target effects. Recent studies indicate that apart of the high effectiveness on the target species, *Lysiphlebus testaceipes* for instance has become the predominant parasitoid of a number of indigenous aphid species, either pests or indifferent species, in all environments from agro-ecosystems to climax ecosystems like forests (Stáry et al., 1988a, b) across the Mediterranean area (Nicoli and Burgio, 1997). The successful introduction of *Cales noacki* as a classical biological control agent against the woolly whitefly *Aleurothrixus floccosus* (Homoptera: Aleyrodidae) may have led to the complete displacement of all competing parasitoids in populations of the non-target whitefly species *Aleurotuba jelinekii* in some parts of the Mediterranean area (Viggiani, 1994). Although both are excellent control agents against their respective target pests, new releases of these agents should be reconsidered and for the time being not be disseminated commercially into areas where they do not yet occur.

The data presented in this paper do not allow us to draw general conclusions about risks for certain guilds of natural enemies (e.g. predatory insects, predatory mites, hymenopteran or dipteran parasitoids) as the chosen species only represent a small selection of natural enemies used in inundative biological control. Extending the methodology to many more cases could lead to important generalisations concerning risk posed by certain groups of natural enemies.

The risk assessment methodology allows for ranking of natural enemies that are evaluated for control of the same pest. For example, for control of whitefly one would conclude that it is safer to use the exotic parasitoid *Encarsia formosa*, than *Encarsia pergandiella* or *Chrysoperla carnea*.

In present day biological control, governments that rigidly regulate the introduction of biological control agents, like for example Australia and New Zealand, usually require that candidate agents undergo host-range testing to ensure that they will not become pests or threaten desirable species (Charles, 2001). This results in a general preference for highly specific natural enemies, as was already common sense among most biological control workers. The commercial biological control industry, however, often prefers generalist, less host specific natural enemy species. Although these species usually get high risk indices (see Table 5: *Cales, Chrysoperla, Delphastus, Encarsia pergandiella, Harmonia, Hippodamia, Lysiphlebus, Orius, Podisus, Trichogramma*), these generalists should not always be excluded from being introduced. The likelihood of adverse ecological effects of these species may be high, but the conditions under which they are released (e.g. greenhouses in temperate climates) may strongly limit the realisation of these adverse effects. An effect that also is surfacing as a result of regulation, is that it encourages the evaluation of native natural enemies as potential biological control agents first; a development that we strongly support. During the process of collecting and evaluating native natural enemies, the potential native host insects will become known which will help later to identify species to be tested for non-target effects.

Implementation of a registration procedure for natural enemies is currently a topic of hot debate among the biological control industry and regulators. The biological control industry foresees lengthy, cumbersome procedures leading to high costs, and thus, in some cases, the possibility that potentially interesting natural enemies will not enter the market because costs are too high. Regulators within ministries of environment and agriculture want to prevent unnecessary and risky releases of exotic organisms. The history of arthropod biological control shows that very few mistakes have been made until now (Lynch et al., 2001). This is a point in favour for the biological control industry, and is in strong contrast with the problems that have been created by accidental importation of pests and diseases on infested plant material by others. The current work by, among others, the EU-ERBIC project will hopefully result in a light and harmonised registration procedure that is not prohibitive for the biological control industry and will result in the pre-selection of safe natural enemies. The purpose of such a registration procedure would be to keep biological control a respected, reliable and sustainable control method, and to prevent import and release of unsafe natural enemies.

The risk assessment procedure proposed in this paper is a step towards a registration procedure. With this risk assessment procedure, biological control experts will be able to put the natural enemies under evaluation for release into different risk categories, which makes is possible (1) to choose the safest control agent when more candidates are available, (b) to decide if more, and what kind, of information is needed for a full risk assessment, and (c) to conclude that certain natural enemies are not suitable for release.

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