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Evaluating indirect ecological effects of biological control

An international Symposium of the Global IOBC, Montpellier, France,
17-20 October 1999

Evaluation des effets écologiques indirects de la lutte biologique

Symposium International de l'OILB Mondiale, Montpellier, France,
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Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles
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L'OILB, Organisation Internationale de Lutte Biologique contre les animaux et plantes nuisibles, a pour but de promouvoir et coordonner les recherches et les applications dans le domaine de la lutte biologique et intégrée. Elle est affiliée à l'Union Internationale des Sciences Biologiques (UISB) et est dotée du statut de liaison de l'Organisation des Nations Unies pour l'Alimentation e l'Agriculture (FAO).

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Contents

Foreword

Biological control has become today a key component of crop protection worldwide. Concern about reliance on chemical pesticides has led to development of integrated pest management, which depends on both the conservation of local natural enemies and their mass release as alternatives to chemicals. Classical biological control, the introduction of exotic biological control agents to permanently suppress exotic pests, is practiced ever more widely as successes accumulate, and as new exotic pest problems follow trade liberalization.

The capacity of introduced natural enemies to persist in the environment, reproduce and spread gives biological control its unique advantage as a pest control method. It also binds the practice of biological control to the science of ecology, through which it can be understood, and it identifies an element of risk through indirect effects of new natural enemy populations on local communities and non-target species.

Biological control has been developed over the past century as an agricultural technology. Not surprisingly, research on non-target effects has focused for many years on risks to crop species and agroecosystems. Placed next to chemical pesticide alternatives, potential environmental risks of biological control have usually seemed negligible. Recent concern about the impact on biodiversity and natural ecosystems of alien species has altered this perception. Growing interest in biological control has drawn in new stakeholders, including conservationists, regulators, policy makers and the general public, who want to be confident of its environmental safety. This greater accountability of biological control to a wider community is the price of its success, and biological control practitioners now have a clear challenge to demonstrate the environmental safety of natural enemy introductions.

This Symposium addresses this challenge by bringing ecologists and practitioners together (about 150 from 22 countries) to develop an understanding of indirect ecological effects of biological control and a scientific methodology for their measurement. A scientifically sound methodology for investigating indirect effects is the first and the most important step in developing responsible and meaningful procedures for risk assessment and decision making in biological control introductions.

This significant meeting is taking place under the aegis of IOBC, Agropolis and C.I.L.B.A. This is no coincidence as IOBC represents a global association of biological control practitioners and C.I.L.B.A. (as part of Agropolis), represents a strong biological control presence of more than 120 practitioners from French, Australian and American institutions. This biological control expertise is concentrated at the International Biological Control Campus in Montferrier-sur-Lez, southern France. It is our hope that this meeting will provide the biological control community with a practical, workable plan for increasing the transparency and accountability of scientific evaluations of indirect ecological effects of biological control. This will enable biological control to remain a viable option for use in sustainable agriculture in the next millenium.

The contributions published in this volume range from theoretical studies on the prediction of indirect effects to novel, practical studies on measuring these effects in the biological control of invertebrates, weeds and plant diseases. They provide a library of focused ecological thought and experimental techniques for assessment that makes this volume immediately valuable to anyone involved in biological control today. Readers will also find through in this volume contacts with a truly global community of researchers currently investigating indirect ecological effects. The studies reported here form the foundation of a collaboration amongst these scientists which will begin at this Symposium with discussions and working groups on methodology, and lead in future to a sounder scientific basis for evaluating benefits and risks of biological control to agriculture and environmental conservation.

Jeff Waage, President, Global IOBC

Alan Kirk, President, C.I.L.B.A.

Effect of *Metarhizium anisopliae* (*flavoviride*) var. *acridum* (Deuteromycotina: Hyphomycetes), on *Neoseiulus idaeus* (Acari: Phytoseiidae), *Clavigralla tomentosicollis* (Heteroptera: Coreidae) and *Orius albidipennis* (Heteroptera: Anthocoridae)

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The entomopathogenic fungus *Metarhizium anisopliae* (*flavoviride*) var. *acridum* Gams & Rozsypal is an efficient biological agent for grasshopper control (Lomer *et al.*, 1997). In 1996 and 1997, the pathogenicity of this fungus on three non-target arthropods was investigated in Benin. The biotests included two beneficials, *Neoseiulus idaeus* Danmark & Musa (Acari: Phytoseiidae), a biological control agent of Cassava green mite, and *Orius albidipennis* (Reut.) (Heteroptera: Anthocoridae), a predator of aphids and mites, and one pest of cowpea, *Clavigralla tomentosicollis* Stal. (Heteroptera: Coreidae).

The effect of *M. anisopliae* on *N. idaeus* was tested using a permanent exposure method by feeding them on eggs of two spotted spider mite *Tetranychus urticae* Koch mixed with dry conidia. The behavior, mortality and the sporulation rate were assessed in a first experiment during 8 days and in a second experiment during 14 days. In both experiments, the number of eggs and nymphs produced by the phytoseiids were assessed during 8 days after exposure. No adverse effects of *M. anisopliae* on the phytoseiids were observed in both bioassays.

To test the effect of *M. anisopliae* on *O. albidipennis* and *C. tomentosicollis,* adult bugs were exposed to spray residues on strips of millimeter paper. The average mortality after 21 days was not significantly different among the different treatment groups, i.e.

(1) *M. anisopliae,*
(2) blank solution (vehicle control),
(3) untreated control.

No sporulation of the fungus was observed in any of the species tested. The bioassays provided further evidence of the narrow host range of *Metarhizium anisopliae (flavoviride)* var. *acridum.*

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Mass releases of *Trichogramma brassicae* Bezd. (Hymenoptera: Trichogrammatidae) against the European Corn Borer in Switzerland: do they pose a risk to non-target butterflies?

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Trichogramma species are polyphagous egg parasitoids currently being used worldwide as inundative biological control agents on several million ha to prevent economic losses in a range of crops. In Northern Switzerland *Trichogramma brassicae* Bezd. (Hym.: Trichogrammatidae) is used for mass release against the European Corn Borer at rates of about 120,000 females per ha, thereby successfully controlling the target pest. However, some dispersal into surrounding habitats must occur and depending on host finding and host acceptance of lepidopteran species present in the habitats, these mass releases may pose a risk to a range of non-target butterflies. In Switzerland, 120 out of the 180 native butterflies are already endangered (named on the red list) and the present study aims at looking for potential detrimental effects of *Trichogramma* mass releases on populations of these butterflies.

We first evaluated which currently endangered butterflies lay their eggs in the period when *Trichogramma brassicae* is released. *T. brassicae* is active in maize fields from mid June to the end of July, a period that overlaps considerably with the egg laying periods of many butterflies. In addition, we checked whether the habitats of these butterflies overlap with the maize-growing area in Switzerland. Only a few endangered butterflies are distributed exclusively within the areas where *T. brassicae* might be released but many more species have overlapping distributions. The risk was ranked for these species according to how they are temporally and spatially exposed to the biological control agent. Host specificity tests were then carried out to determine whether *T. brassicae* can attack and develop within butterfly hosts that are potentially exposed to this parasitoid. Eggs from endangered *Arghynnis adippe* Denis and Schiffermüller, *A. niobe* L., *Melicta athalia* Rottemburg (Lep.: Nymphalidae) as well as *Plebejus idas* L. (Lep.: Lycaenidae) were readily accepted by *Trichogramma* females in the laboratory. The same holds true also for other species not yet named on the red list such as *Papilio machaon*, L. (Lep.: Papilionidae), *Pieris napi* L. (Pieridae), *Melanargia galathea* L. (Lep.: Satyridae) and *Cyaniris semiargus* Rottemburg (Lep.: Lycaenidae). Despite the fact that development of *Trichogramma* offspring - as in all other species mentioned above – was possible, very few eggs of the endangered *Melitaea parthenoides* Keferstein (Lep.: Nymphalidae), of *Coenonympha pamphilus* L., *Pararge aegeria* L. (Lep.: Satyridae), *Hesperia comma* L. (Lep.: Hesperidae) and an as yet undetermined geometrid were accepted for oviposition by the biocontrol agent. We also tested two sphingids, *Deilephila elpenor* L. and *Sphinx ligustri* L., and found that parasitization success was poor although females tried to penetrate the thick chorion extensively.

We have shown that *T. brassicae* attack and can develop within a range of butterfly hosts including species named on the red list while others are significantly less suitable than the target pest. Further choice tests and field exposure experiments are under way to further elucidate the impact of these mass releases.

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Evaluation of non-target effects of native and introduced entomopathogenic nematodes

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In the United States, interest in the use of nematodes in the families Steinernematidae and Heterorhabditidae as biological control agents is growing rapidly. Commercial products are being promoted in a wide variety of pest control markets including ornamental and nursery, homeowner/urban, turf, plus perennial and annual agriculture. We are studying some of the non-target impacts associated with the application of introduced and native EPNs. Here we report results from a three-year field trial to test the effects of introduced EPNs on endemic EPNs and non-target arthropods in the soil.

The introduced EPN, *Steinernema riobrave*, was applied once each year to conventionally tilled and no-till corn fields that contained endemic populations of *S. carpocapsae* and *Heterorhabditis bacteriophora*. Soil samples were periodically collected for detection of EPNs using the *Galleria* bait method. Arthropods and soil nematodes were also extracted from a portion of the soil samples.

Both the introduced and native nematodes were detected throughout the growing seasons. *Steinernema riobrave* was not detected in any plots in which it had not been applied in 1997, but was detected twice outside of treatment plots in 1998. In 1998, *S. riobrave* was detected in plots that had been treated in 1998. The infection rate of native and introduced EPNs depended on tillage. The total number of insects infected by native EPNs was higher in no-till than in conventionally tilled plots. Non-native *S. riobrave* and native *H. bacteriophora* were insensitive to the conditions created by conventional tillage in comparison to *S. carpocapsae*. The insensitivity of *S. riobrave* to conditions created by tillage may favor its persistence at higher levels than native *S. carpocapsae* in tilled soils.

The application of *S. riobrave* was associated with a non-significant reduction in numbers of insects infected by *S. carpocapsae* and *H. bacteriophora*. The detection of multiple species from a single core was rare relative to the number of cores from which only one nematode species was detected. There was a significant negative association between *S. carpocapsae* and *S. riobrave*. *S. carpocapsae* and *S. riobrave* were detected together in only 1.5% of the cores, and *H. bacteriophora* and *S. riobrave* were detected together in only 0.4% of the cores. There were no cores in which all three nematodes were detected together, and no insect cadavers infected by more than one nematode species. This negative spatial association could help explain the short-term coexistence of the native and introduced arthropods. There may be some behavioral or other mechanism which reduces spatial overlap and could allow an introduced EPN to establish without leading to the complete displacement of native species. Patchiness of EPN distribution is probably favorable for coexistence of multiple species at a site.

Application of EPNs affected total numbers of microarthropods and mites, but not Collembola. Numbers of microarthropods other than mites and Collembola were greater in no-till and conventionally tilled plots treated with *S. riobrave* compared with the control or *H. bacteriophora*-treated plots. Numbers of mites were lowest in *H. bacteriophora*-treated plots. Non-target macroarthropod numbers were lower in nematode-treated compared to untreated plots. We are currently processing data collected from similar field experiments in 1999. The significance of arthropod community changes associated with application of entomopathogenic nematodes will be assessed after identifications are completed.

Potential for impact of *Microctonus* spp. (Hymenoptera: Braconidae) outside the target host environment

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Two species of *Microctonus* (Hymenoptera: Braconidae) have been introduced into New Zealand for biological control of adult weevil (Coleoptera: Curculionidae) forage pests. *Microctonus aethiopoides* Loan was released in 1982 to control *Sitona discoideus* Gyllenhal in lucerne, and *M. hyperodae* Loan in 1991 to control *Listronotus bonariensis* (Kuschel) in ryegrass. Post-release studies have shown that *M. hyperodae* has demonstrated almost complete host specificity (Barratt *et al.*, 1997) as predicted by pre-release quarantine studies (Goldson *et al.*, 1992). In comparison, *M. aethiopoides* has a relatively broad host range, attacking a number of non-target species both in the laboratory and field (Barratt *et al.*, 1997).

In this contribution, methods of investigating potential impacts of these parasitoids on non-target species, particularly outside the target host environment will be discussed. This includes consideration of the potential for parasitoids to exploit 'new' environments, identification and distribution of taxonomically and ecologically susceptible hosts, and comparative phenology of target and non-target hosts. Indirect effects such as the potential for competition or displacement of native *Microctonus* species will be considered.

Sampling has shown that both *S. discoideus* and *L. bonariensis* can be found in habitats quite distant from the agricultural environment, in sub-alpine to alpine predominantly native vegetation. Grassland sites progressively distant from target host environments have been sampled which indicate that *M. aethiopoides* parasitises native and other non-target weevil species in habitats where non-target hosts and particularly target hosts are present at relatively low densities compared with the more intensive agricultural environment. Questions relating to the possibility that *M. aethiopoides* populations are sustained by non-target weevils distant from the host environment or whether native species are attacked by parasitoids which have originated from *S. discoideus* populations will be discussed.

The objectives of a recent field release of *M. aethiopoides* in a sub-alpine grassland area where it is not currently established, and where pre-release native weevil population density data are available for a number of years, will be described. This experiment is an opportunity to determine the potential for *M. aethiopoides* to establish at a site quite distant from target host populations, and if it does, to measure the impact on native weevil population densities over a number of years.

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Predicting trophic interactions

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Scientists, starting with A.J. Lotka and Vito Volterra in the 1920s, have developed ideas and models to explore the interactions between species. This was despite C. Juday's criticism during the late 1890's, that mathematics could not be used to represent these interactions because it was too based on the assumption of uniformity when nonuniform factors were involved. The earliest models were homogenous in respect to space and time the majority still is (see Bax, 1998, for review). Lotka, in fact developed his ideas on the basis of an analogy between biological populations and homogenous chemical systems. In these early homogenous models and their derivatives generalist predators were stabilizing forces.

However, predation experienced by a population is influenced by environmental and biological factors that vary spatially, seasonally, annually, and with the abundance of itself, other prey species and the predator species. Predation can directly regulate prey numbers and indirectly regulate their survival via habitat availability, individual growth and trophic structure. Stabilizing predator-prey features include: refuges, invulnerable classes of prey, resource limitation for the prey, and spatial heterogeneity. Destabilizing features include time lags (eg. predators with longer life spans than their prey) and trophic complexity.

Recent work has placed greater emphasis on the role of spatial heterogeneity in promoting stability non-equilibrium dynamics at the local population level may produce equilibrium dynamics at the metapopulation level. Local extinction may favour overall system persistence and, paradoxically; generalist predators may be forces for stability by causing local extinction. The scale of the regions of stability and instability depend on the degree of dispersal of offspring. Some difficulties in identifying regulatory mechanisms for populations, even though their presence is required to explain the long-term persistence of many populations in nature, may derive from looking at the wrong scale. This could explain why, despite the emphasis on population regulation by ecologists over the last 50 years, we are still no closer to a general solution (Murdoch and Bence, 1987). However, emphasis over the years has changed from a concern with equilibrium, homogeneity, determinism and local or single-level properties of systems to nonequilibrium, heterogeneity, stochasticity and hierarchical properties of ecological systems. Future discoveries in population dynamics may come from considering the richness of natural communities. Experimental approaches may need to be intrinsically linked with large scale management programs to work at the scales that are relevant to system stability, *ie.* adaptive management.

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Effect of *Harmonia axyridis* (Coleoptera: Coccinellidae) invasion on the aphidophagous coccinellid guild on apple in West Virginia, USA

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Harmonia axyridis was first seen in apple orchards of West Virginia, USA, in 1994. By 1995 it had become the dominant aphidophagous coccinellid, displacing *Coccinella septempunctata*, which has been present in the region since 1983. These two introduced coccinellids have comprised over 70% of the aphidophagous coccinellid fauna on apple since 1989. Native aphidophagous coccinellids have been rare in apple orchards since the arrival of *C. septempunctata*. However, *Coleomegilla maculata lengi* abundance increased since the appearance of *H. axyridis* in 1994 (Brown & Miller, 1998). This recent increase in *C. maculata lengi* led to the hypothesis that by displacing *C. septempunctata*, *H. axyridis* allows for increases in populations of native coccinellids. Intensive sampling in 1999 showed that *C. septempunctata* comprised about 50% of the adult coccinellids on apple. *Harmonia axyridis* was nearly as abundant as *C. septempunctata* and adults of *Adalia bipunctata*, *Cycloneda munda*, *C. maculata lengi*, *Hippodamia convergens* and *Chilocorus stigma* were also seen in apple orchards. These observations support the hypothesis that the presence of *H. axyridis* is allowing a return of some native coccinellids into apple orchards. However, about 90% of all larval coccinellids on apple are *H. axyridis*.

In addition to affecting the coccinellid guild, the arrival of *H. axyridis* has negatively impacted at least one other aphid predator, the cecidomyiid (Diptera) *Aphidoletes aphidimyza*. Prior to the appearance of *H. axyridis*, *A. aphidimyza* was the most abundant aphid predator (Brown & Lightner, 1997). By 1997, *A. aphidimyza* populations had declined to about 35% of their 1996 levels.

In 1999, only one aphid colony was found to have *A. aphidimyza* present. Both aphid infestations and the average length of time a single colony survives have been reduced since the arrival of *H. axyridis*. Historically, *A. aphidimyza* does not appear in aphid colonies until after they begin to grow exponentially. The early predation of aphid colonies by *H. axyridis* and *C. septempunctata* has removed aphids on apple as a resource for *A. aphidimyza*. The decrease in *A. aphidimyza* populations on apple could impact other crop systems that may rely on dispersal of this predator from apple in mid-summer.

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Assessing indirect effects of plant pathogens for biological control of weeds

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The main concern of regulators, the public, and scientists, about plant pathogens and insects for biological weed control is non-target damage. For the vast majority of agents, the first step in evaluation is a pre-release risk-assessment conducted under containment greenhouse and laboratory conditions. This resolves most issues of negative indirect effects. However, the topic for this paper is to review the approaches used under more natural conditions.

Formalized field plot studies have been used in some cases to strengthen data on the safety of plant pathogens for weed control. Some confusion has resulted from direct inoculations of non-target species in the field. In these instances, plant reactions were similar to those from artificial greenhouse studies (Bruckart *et al.*, 1996, Hasan *et al.*, 1989), compared with a lack of non-target infections when plants are subjected to more natural levels of inoculum (Baudoin *et al.*, 1993).

An epidemiological model was used to clarify the risk of using *Chondrostereum purpureum* as a mycoherbicide in the Netherlands. Data indicated inoculum produced from treated tree stumps would not significantly raise the numbers of spores already present from natural infections (deJong *et al.*, 1990), and therefore risk was determined to be very low.

Opportunities to measure indirect effects also result from unanticipated discovery of pathogens in new areas. Following introduction of *Phragmidium violaceum* into Australia, a survey of *Rubus* spp. was made to determine susceptibility, spread, and need for additional agents (Bruzzese & Field, 1985). Increasing interest in post-release follow-up studies, long-term ecological effects, new molecular tools, and better modeling, will further improve knowledge and understanding of biological control, its risks and benefits.

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Introduction of *Trichopoda giacomellii* (Diptera: Tachinidae) as a biological control agent for *Nezara viridula* (Hemiptera: Pentatomidae) and its potential for impact on the non-target hosts *Plautia affinis*, *Glaucias amyoti* and *Alciphron glaucus* (Hemiptera: Pentatomidae)

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The tachinid parasitoid, *Trichopoda giacomellii* (Blanchard) was approved for release in Australia in 1996 as a biological control agent for the green vegetable bug *Nezara viridula* (L.). Releases of *T. giacomellii* occurred over three years at sites in western New South Wales and southeast Queensland and is now confirmed as established in these regions. Quarantine studies (Sands & Coombs, 1999) identified three indigenous pentatomid bugs (*Plautia affinis*, *Glaucias amyoti* and *Alciphron glaucus*) as supporting complete development of the agent, two of which are known to occur in habitats in common with *N. viridula*. *Plautia affinis* is a minor pest of agricultural crops whereas *G. amyoti* and *A. glaucus* are apparently forest adapted species with no pest status.

Population densities and parasitism rates of *N. viridula*, of *P. affinis* and *G. amyoti* were measured at one release site in western New South Wales during 1999. All three species were found feeding on fruits of the introduced weed *Ligustrum lucidum* Aiton (broadleaf privet) during the months of February to June. Peak seasonal abundance of *N. viridula* occurred earlier (Feb/Mar) than that of either *P. affinis* or *G. amyoti* (April/May). Parasitism rates averaged 9.1% (75/820) of *N. viridula* adults, < 1% (2/222) of *P. affinis* adults and < 1% (3/410) of *G. amyoti* adults. Parasitised *P. affinis* and *G. amyoti* adults were recovered only when *N. viridula* population numbers were highest on broadleaf privet. Late in the season, when *N. viridula* was either low in abundance or absent, no parasitised *P. affinis* or *G. amyoti* were recovered.

Recovery of parasitised *N. viridula* from the host plant *Ricinus communis* (L.) (castor oil) indicated parasitism levels of between 9-73 % during January to June 1999. Castor oil does not support feeding of either *P. affinis* or *G. amyoti*. These results suggest that although *T. giacomellii* was active throughout the study period, the non-target hosts *P. affinis* and *G. amyoti* were parasitised only when they co-occurred with *N. viridula* on broadleaf privet. *Plautia affinis* is also known to feed on a range of other crop and weed hosts with *N. viridula*. Parasitism of *P. affinis* by *T. giacomellii* may also occur on these plants. Alternative (native) hosts for *G. amyoti* are not known.

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Introducing European parasitoids of tortricid grape berry moths into North America: evaluating the potential for a program in the U.S.

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The native North American grape berry moth *Endopiza viteana* (Clemens) (Lepidoptera: Tortricidae) is a pest of grapes in the eastern U.S. There is interest in possible introductions of European tachinid parasitoids of the European grape berry moths *Lobesia botrana* (Denis & Schiffermüller) and *Eupoecilia ambiguella* Hübner against this pest, because the natural enemy niche occupied by tachinids in Europe appears to be unfilled in North America. The first step in evaluating the potential value of such introductions, preceeding any environmental assessment in the U.S., is to determine whether any of the 5 recorded European parasitoids have significant impact upon European torticid berry moths, to determine the accuracy of host range data *in lit.*; and to assess the impact these species have on the alternate host species in Europe. There are 4 species of tachinids reported from *L. botrana* and 2 from *E. ambiguella* (one species attacks both tortricids). Alternate plant hosts such as *Daphne gnidium* (Thymeleaceae), a native shrub in the Mediterranean region thought to be the original host plant of *L. botrana*, would be logical alternate hosts on which to begin an ecological study of the relationship between the natural parasitoids and their tortricid hosts.

Concurrently in the U.S., a broad initial assessment of potential non-target effects on closely related tortricids such as, *Argyrotaenia velutinana* Fernald, *Choristoneura rosaceana* Harris, *Cydia pomonella* (L.), *Platynota idaeusalis* (Walker) and *Grapholitha molesta* (Busck) will be conducted. Once these steps have been completed, if any of the European tachinids appear promising, a refined list of potential non-target species in the U.S. will be compiled for further assessments.

Ecological aspects of using micro-organisms to control plant diseases, and possible non-target effects

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Plant-associated bacteria hold great promise for the biocontrol of soilborne pathogens. Understanding how these bacteria interact in their environment is important for optimizing strain performance. Ecological studies are also an essential step in assessing inoculant biosafety after environmental release. We will present an ecological overview of antagonistic bacteria in soil habitats, using the biocontrol strain *Pseudomonas fluorescens* CHA0 as a model. Synecology studies examined horizontal transfer of chromosomal genes in the rhizosphere, target effects on pathogens, and non-target impact on native micro-organisms. Autecology studies examined the persistence of introduced inoculants in both active and viable-but-not-culturable states, and bacterial spread through the soil profile and into groundwater. The relative impact of wild-type biocontrol inoculants was compared with genetically-modified strains and with other cultural practices. We determined the ecological role of several antimicrobial compounds and global regulatory genes. Methods suitable for biosafety assessment will be highlighted, particularly molecular approaches. Recent work studying the ecology of microbial inoculants in the non-target habitat of crop residues will be discussed.

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Expanding and documenting ecological research in classical biological control programs

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One of the key criticisms about classical biological control (e.g., Howarth, 1991, Miller and Aplet, 1993, Simberloff and Stiling, 1996) is the perceived lack of a sound scientific basis for some of the ecological research that has long been the foundation of programs. For example, host-specificity testing has been singled out as needing a stronger ecological basis, and post-release monitoring has been criticized as being the weakest part of the biological control process because of the way it is planned, funded, conducted and reported.

Many of the criticisms of biological control are unfounded, based on a lack of understanding of the process, misinterpretation of reported results, or other factors. However, some of the criticisms are valid. It is an ethical responsibility for biological control researchers to address objectively the criticisms, review current procedures and make changes that will result in better science, ultimately altering the perception of biological control by objective observers.

The U.S. Department of Agriculture, Agricultural Research Service, continually assesses biological control as part of integrated pest management programs. Key changes in the ARS project planning system are being implemented that will improve the accountability and documentation of biological control. These changes include: requirement for a long-term program plan, including greater emphasis on testing refutable hypotheses and on post-release monitoring of impacts of biological control agents on the target and on potential non-target species; incorporation of cultural control/revegetation; and increasing partnerships.

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Retrospective case studies to test a protocol for predicting host range of parasitoids introduced for biological control

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Biological control by introduction of natural enemies is increasing in Brazil and other countries in the southern cone of South America. Although regional standards based on the FAO code of conduct for such introductions have been approved, they lack detail about how to evaluate potential host range prior to introduction. Thus, a detailed protocol is a needed for this region. Here we outline a protocol for such evaluation and then test it with retrospective case studies on *Macrocentrus cingulum* and several other parasitoid species introduced for control of insect pests in North and South America. The protocol involves analysis of the host range in the area of origin (using literature and field studies), biological characteristics of the natural enemy, and quarantine assessment of physiological/behavioral host acceptance/suitability of species in the area of introduction. This information is used to predict ecological/evolutionary host range in the area of introduction. We discuss problems raised during these case studies and ways the problems could be overcome in developing countries.

Methodologies for studies of interactions between hosts and pathogens in the insect-pathogenic fungal genera *Entomophthora* and *Strongwellsea*

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Entomopathogenic fungi from the genera *Entomophthora* and *Strongwellsea* (Zygomycota: Entomophtorales) cause natural epizootics among insect pests in agriculture and husbandry. They have importance for natural population regulation and possess potential to be developed as biological control agents. We have studied the interactions between species from these genera and their hosts by means of the following methods: field sampling and prevalence assessment, morphological diagnosis, *in vivo* and *in vitro* growth, transmission between host species, and molecular characterization.

Species from the genus *Entomophthora* affects insects from several orders: Diptera, Hemiptera, Thysanoptera, Hymenoptera, Neuroptera and Coleoptera. Each described species from *Entomophthora* does only occur in one insect host order, but on host species from several families. Laboratory transmission of *Entomophthora* spp. between dipteran hosts and from Hymenoptera to Diptera is, however, possible. The complete life cycle including the sexual stage of the fungi is only documented in certain hosts. We conclude for *Entomophthora* spp. that development of epizootics can be a result of several host and pathogen species interactions. The host species can be from different dipteran families or even the more taxonomically distant.

Species from the genus *Strongwellsea* have only been documented on adult dipteran hosts from a limited number of families including Anthomyiidae, Fanniidae and Muscidae. *Strongwellsea* is much more restricted with respect to ecological and physiological host range than *Entomophthora* and laboratory transmission of disease was only possible between taxonomically closely related host species. Concerning *Strongwellsea* we conclude that development of epizootics is the result of a one host, one pathogen population interaction or it may involve a few taxonomically very closely related hosts.

Our studies document the importance of studying simultaneously both host and pathogen ecology in the natural habitats. The studies further emphasize the necessity of clarifying the ecological host range, including morphological and molecular methods for characterization and for testing hypotheses on ecological host range by performing relevant host range studies in the laboratory.

Indigenous and exotic parasitoids: competitive displacement or complementary action?

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Interspecific competition of parasitoids for the same host results in competitive displacement (exclusion) of one species by another or in complementary action which tends to increase the total degree of biological control (DeBach, 1966).

This contribution presents two cases of classical biological control, by introduction of exotic parasitoids: the cassava mealybug (*Phenacoccus manihoti*) in Congo and the citrus purple scale (*Lepidosaphes beckii*) in New Caledonia.

In the first case, the exotic parasitoid (*Epidinocarsis lopezi*), which was introduced to control the cassava mealybug populations (seasonal outbreaks), has caused the competitive displacement of a local species of *Anagyrus* which have coexisted with it for a time but which can no longer be found in the cassava fields. The same mechanism was observed in Gabon where indigenous species *Aphytis cochereaui* is able to maintain itself on low levels of the host population and prove active at the very beginning of the gradation of the scale, whereas *Aphytis lepidosaphes*, the exotic species, intervene more efficiently but later in the course of the outbreak.

In New Caledonia, where one finds traditional citrus orchards under shade and citrus groves in the open, this mechanism of complementary action is softly modulated according to the kind of habitat and the environmental conditions (biotic and abiotic). A similar situation might be observed in the future in Congo, under the "false rubber" culture conditions (hybrid of *Manihot esculenta* and *M. glaziovii*), for recent investigations have shown the population of the mealybug is numerically more stable and the entomophagous fauna of the host more diversified.

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The comparison of pests and natural enemies in their areas of origin and introduction: the scope and value of extensive ecological studies in the broom biocontrol programme

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Biological control programmes have varied widely in the extent of ecological work undertaken on potential agents and their target organisms. At one extreme are biocontrol programmes where native range work has been confined to surveys for potential agents, followed by their shipment to the target country and release. Modern biological control programmes require at least that the potential biological control agents are tested to assess the risk of attack on non-target organisms. There is also increasing emphasis on assessing the impact of biocontrol agents on the target organisms after release, and in future this is likely to be expanded to include direct and indirect effects on non-target organisms. In the field of weed biocontrol, because of the potential risk to useful plants from introduced herbivores or diseases, host specificity testing was often a feature even of programmes undertaken a considerable time in the past.

One modern weed biocontrol programme, against Scotch broom (*Cytisus scoparius*), can be used as an example of the other extreme in biological control, where very extensive studies have been conducted in the native and exotic range of the weed. These studies are reviewed, including the classic insecticide check experiment that ran for 11 years at Silwood Park in southern England, the numerous PhD programmes investigating the ecology of insect herbivores on broom, and the more recent ecological and host range studies as part of the biological control programme against broom. The sum of ecological knowledge of broom as a native plant and as an introduced weed is now considerable, and allows us to be more confident that classical biological control can play a role in the management of this invasive alien weed in countries such as New Zealand, Australia and the USA.

Bodies such as the new Environmental Risk Management Authority in New Zealand now require a risk assessment approach to assessing biocontrol releases: without evidence that the released agent should impact the target weed (a positive benefit) there will be no approval for release no matter how small the perceived risk to the environment may be (a risk of negative effects). The host specificity testing of potential broom biocontrol agents allows the risk of attack on non-target plants to be assessed. Several otherwise promising potential biocontrol agents have been rejected after failing such tests, and major conflicts of interest still remain with non-target introduced plants perceived of value in New Zealand and Australia, and with native plants species in the same tribe as broom in the USA. Finally, the cost in time and resources of the extensive ecological work and safety testing on broom is estimated. If such a model is the way forward, then biological control programmes will have to become still better resourced and their sponsors even more patient.

Effect of host plant on Brassicaceae specialist / generalist aphids and on their natural predator, *Adalia bipunctata* L. (Coleoptera: Coccinellidae)

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Secondary plant substances, called allelochemicals, play a major role in pest infestation. Glucosinolates and their degradation products (mainly isothiocyanates, ITC) are powerful stimulants for Brassicaceae herbivores but deter the non crucifer feeders. These plant compounds are tolerated by the generalist *Myzus persicae* Sultzer by ignoring or avoiding them. Thioglucosidases enzymes capable of releasing ITC from glucosinolates were found in the specialist *Brevicoryne brassicae* L. Do these substances have an effect on the aphid predators ?

Aphidophagous Coccinellidae are known to be polyphagous to a wide range of aphid species even if only a limited number of species provide suitable food for *Adalia bipunctata* L. *Brassica napus* and *Sinapis alba* were used as aphid host plants. While both specialist / generalist aphids were positively influenced by Brassicaceae species, mixed effects are recorded in ladybird performances following the aphid species / host plant combinations. Developmental (larval mortality, adult weight and developmental durations) and reproductive parameters (fecundity, egg viability) were observed. Significant differences appeared according to aphid host plant and aphid species. This kind of chemical ecology studies will enhance a better understanding of interactions between plant and insects. The plant - aphid - predator tritrophic model was used to suggest improvements in pest biological control.

The occurrence of *Rhinocyllus conicus* on native North American *Cirsium* species: was it predictable from pre-release studies?

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Host specificity tests with *Rhinocyllus conicus* populations from *Carduus nutans* showed no preference between *Carduus* and *Cirsium.* Comparisons of sampled populations of *R. conicus* from all regions of western and Mediterranean Europe suggest a separation into biotypes based on regional climate types such as: 1) a Mediterranean-climate group using, *Silybum marianum* but also having an affiliation with genus the *Carduus*, 2) a continental-climate group restricted to the genera *Carduus* and *Cirsium*, and 3) an oceanic-climatic group specializing on *Cirsium* in the field. There is a strong affinity for both *Carduus* and *Cirsium* hosts in *R. conicus* from *Carduus* spp. The basis for host-utilization patterns depends on the synchrony of potential host plants within the thistles, with a strong preference for a particular species in any given area. High dispersal capability is indicated by the extended distribution of the weevil in Europe and its high rate of occurrence at thistle sites.

Experience and evaluation of non-target effects of pathogens used for management of arthropods

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Entomopathogens can be used in classical, conservation and augmentative biological control. For the most part, entomopathogens have been developed as microbial insecticides and used for inundative augmentation, although there remains great potential in using them with classical and conservation approaches. Ecological effects of pathogens on non-target organisms can chiefly come about through 1) depletion of the target host population and 2) direct infection of non-target hosts. Although depletion of the target host is usually the desired effect, this can have additional ecological effects nonetheless. However, such effects are usually reversible when inundative biological control impacts indigenous organisms, as effects that have been documented are minimal and pathogen levels eventually return to background levels following application. Irreversible effects could occur if an exotic or genetically engineered pathogen were to become established and provide long-term control, as would be expected in classical biological control for instance. Such ecological effects may be minimal if the target population has been introduced in the first place and if the pathogen is highly host specific. Of course it may be very undesirable if endemic target invertebrates are affected. To date there is little evidence of detrimental ecological effects from deliberate introductions of entomopathogens for use in "classical" biological control. Most studies of detrimental effects of entomopathogens have centered on pathogens being used inundatively, principally with *Bacillus thuringiensis*. Although direct impacts to non-target invertebrates closely related to the target host are common, there is little evidence to suggest that these pathogens become established in populations of non-targets, thereby causing long-term effects. One of the main reasons for reluctance to use entomopathogens in biological control has been that protocols and methods for evaluation of the potential risks of introductions of entomopathogens were lacking. There has also been great reluctance in releasing genetically modified entomopathogens for similar reasons. Protocols for registration and release of genetically modified organisms are becoming available and several such entomopathogens have now been released, with no evidence of detrimental ecological effects. However, such protocols rely almost exclusively on laboratory studies, many of which may provide meaningless information, as far as prediction of long-term detrimental ecological effects is concerned. A better understanding of basic pathogen and target host ecology and epidemiology is needed in order to better predict potential detrimental effects. Long-term studies of entomopathogens already registered and in use could provide models for risk assessment. The development of better and more pertinent evaluation methods and protocols is also needed.

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Assessment of potential adverse effects to non-target trees from the use of *Chondrostereum purpureum* for vegetation management

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Myco-Tech® is a new bioherbicide being developed by Hydro-Québec in Canada to prevent regrowth of undesirable trees in rights-of-way. The control agent is an indigenous strain of the basidiomycete, *Chondrostereum purpureum* (Pers. ex Fr. Pouzar), an early colonizer of fresh wounds on numerous broadleaf species. This fungus causes the occlusion of vessels and foliar damage that occasionally lead to tree mortality. Following its application to the freshly-cut surfaces of target trees and under favorable environmental conditions, numerous basidiospores are released from treated sites. The hazard posed by such artificially increased inoculum to non-target plants has been assessed.

A "maximum challenge concentration" of *C. purpureum* was induced by placing a total of 1700 small paper birch logs, previously infected with Myco-Tech®, evenly over a field test area of 0.6 ha. To assess the impact of the consequent added load of spores, a total of 38 sampling plots, distributed on 8 transects, within 600m of the infection area were established. Within each plot, 10 *Betula papyrifera* (Marsh.) trees were cut to allow exposure to both the fall and spring periods of spores emission. In addition, 35 apple trees were systematically planted within the experimental area and wounded during spore emission. In the two subsequent years, surveys for presence of the fungus were conducted and detection of the deployed fungal strain was carried out, using RAPD markers. The results support a minimal risk scenario to the environment.

Ecological aspects of the survival in soil of spray released *Bacillus thuringiensis* subsp. *kurstaki*

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Bacillus thuringiensis has been used for many years for insect control without reports of adverse effects in the environment. Several studies have focused on the environmental fate of spray released *Bacillus thuringiensis*. Generally, *B. thuringiensis* in the phylloplane have a very low persistence, while *B. thuringiensis* in the soil have a much higher persistence (Hansen *et al*., 1996). In 1993 we performed a field trial with a rifampicin resistant *B. thuringiensis* subsp. *kurstaki* for control of lepidopteran pests (Pedersen *et al*., 1995). 10^4 CFU were sprayed per gram soil. After 1 year, 10^3 spores (CFU) per gram soil were still present.

Since 1993 no ploughing and no fertilization has been performed in the test field. In 1999, six years after the field trial, soil samples were collected and analyzed for rifampicin resistant *B. thuringiensis*. Twenty soil samples collected within one square meter contained from 200 to more than 1000 rifampicin resistant *B. thuringiensis* per gram soil. Although the soil samples were collected after a period of rain and at a growth permissive temperature, no indication of vegetative (heat sensitive) *B. thuringiensis* in soil was observed. The spores we find must either have survived in protected niches, or they are a result of local multiplications.

Generally, little is known about environmental niches for vegetative growth of *B. thuringiensis* and the other members of the *B. cereus* group. Recently we found, that *B. cereus* and *B. mycoides* were present in a vegetative stage (heat sensitive) in earthworm gut, while both bacteria were present as spores in the surrounding soil and in the earthworm feces. Likewise, guts of earthworms collected in the field trial area contained vegetative rifampicin resistant *B. thuringiensis* subsp. *kurstaki*. These findings indicate that earthworms may play an important role for activity and survival of *B. thuringiensis*.

To verify the identity of the rifampicin resistant *B. thuringiensis* found in 1999 with the 1993 spray released rifampicin resistant *B. thuringiensis* subsp. *kurstaki*, 200 of the recovered rifampicin resistant *B. thuringiensis* subsp. *kurstaki* isolates were analyzed by phase contrast microscopy and random amplified polymorphic DNA (RAPD). All isolates contained bipyramidale crystals typical for *B. thuringiensis* subsp. *kurstaki*, and all isolates had RAPD patterns identical with the RAPD pattern of the spray released rifampicin resistant *B. thuringiensis* subsp. *kurstaki*.

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Methodologies for assessing the overwintering potential of non-native arthropods

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The use of non-native arthropods for the biological control of glasshouse invertebrate pests in the UK is an increasing feature of agricultural and horticultural systems. When such arthropods are introduced into the UK, particularly into glasshouses, there are no effective means of preventing their escape into the wider environment. This could lead to the establishment of such arthropods in the UK, if they were able to survive through winter. If the escaped arthropods were to establish, it could result in adverse effects on native populations, such as for instance, predation and parasitism of prey species of conservation importance, or competition with native natural enemy species for the same prey or hosts.

Our work examines the overwintering ability of a number of non-native arthropods which are under active consideration for licensed release for the control of glasshouse pests, including *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) and *Delphastus catalinae* Gordon (Coleoptera: Coccinellidae). The further objective is to develop a laboratory protocol for the routine assessment of winter survival, cold tolerance and developmental threshold of non-native arthropods as part of the licensing system.

Non-target impact of *Rhinocyllus conicus* (Froelich) on thistles native to California and their associated insect fauna

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Rhinocyllus conicus Froelich (Coleoptera: Curculionidae) is a thistle flower-head-feeding weevil introduced into North America from Europe as a biological control agent of adventive thistle species in the 1970's. This oligophagous curculionid was expected to control Italian thistle, *Carduus pycnocephalus* L., and milk thistle, *Silybum marianum* (L.) Gaertner, in California. Soon after its release, the weevil was found feeding on non-target native *Cirsium* thistle species (Louda *et al.*, 1997). Goeden and Ricker (1985) reported the asynchrony of Italian thistle phenology and *Rhinocyllus conicus* development as the primary cause of its being an ineffective control agent in California. We quantified the impact of *R. conicus* on native thistle species and its associations with native insect flower head guild members.

Rhinocyllus conicus exhibited differences in abundance among field study locations. More *R. conicus* were found at mountainous locations with a mean number of 1.05 ± 0.11 in the flower heads of *Cirsium occidentale* (n = 457) and 1.05 ± 0.05 in flower heads of Italian thistle (n = 346) compared to a mean number of 0.59 ± 0.08 and 0.85 ± 0.03, for *C. occidentale* and Italian thistle, respectively, at coastal locations.

The number of *Rhinocyllus conicus* in *C. occidentale* flower heads obtained from mountainous locations were greater than these obtained from coastal locations (T = 3.25 and p = 0.0005). The number of *R. conicus* in Italian thistle flower heads obtained from mountainous locations were greater than those obtained from coastal locations (T = 3.25 and p = 0.0012). Further, greater numbers of *R. conicus* were obtained from Italian thistle flower heads on east-facing slopes than west-facing slopes at our field locations (T = 5.34, p = 0.001).

The difference in *R. conicus* abundance among locations was likely due to micro-climate differences. Although, *R. conicus* caused significant seed reduction in Italian thistle, it did not do so in *Cirsium occidentale.* Italian thistle flower heads, uninfested and infested by *R. conicus,* produced 5.18 ± 0.35 and 1.26 ± 2.28 seeds (T = 10.51, p = 0.001), and *C. occidentale* flower heads, uninfested and infested by *R. conicus,* produced 70.50 ± 10.50 and 54.00 ± 10.00 seeds (F = 1.56, p = 0.214), respectively.

Cirsium occidentale flower development was seasonally asynchronous with *R. conicus* development. Flower head production continued after the peak infestation of *R. conicus.* Thus, more than half of the flower heads examined of *C. occidentale* and *C. fontinale* var. *obispoense* were uninfested by *R. conicus.*

There were very few interactions with native insect thistle flower head feeders and *R. conicus* during our study. Further study is needed to determine the extent, if any of these interactions.

Based on our findings, we believe that *R. conicus* is not detrimental to California native thistles, *Cirsium occidentale* and *Cirsium fontinale* var. *obispoense.*

Why introduce aphidophagous ladybirds?

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Since 1874 there have been 155 introductions of aphidophagous ladybird species (Dixon, *in press*). One of the most recent was the release of about 150,000 *Coccinella septempunctata* in North America. This species is now well established (Schaefer *et al.,* 1987). Although its arrival was warmly welcomed, it does not seem to have reduced the abundance of pest aphids (Elliott *et al.,* 1996). The lack of success is characteristic of all the 155 introductions and there is now a growing concern about the side effects of such introductions (Elliott *et al.,* 1996, Simberloff & Stiling, 1996).

Natural populations of native ladybirds are seen as inefficient, nevertheless alien ladybirds are thought to be more likely to be efficient at controlling pest aphids. This is unlikely, firstly, because there is no field evidence that ladybirds regulate aphid abundance (Dixon, 1998) and secondly, because of the way female ladybirds forage. When searching for oviposition sites, they avoid aphid colonies where conspecific larvae are already present. As a consequence, their numerical response to aphid abundance is weak (Hemptinne *et al.,* 1992, 1993).

To safe-guard the good name of classical biological control, introductions of ladybirds should be restricted to those species that have a developmental time that is similar or shorter than that of their prey, and are prey specific (Dixon, in press). On a positive note augmentative biological control using native ladybirds has been more successful. Effectiveness in this form of biological control would be greatly improved if cheap methods of producing ladybirds could be developed and we had a better understanding of ladybird ecology.

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Evaluation of non-target effects: comparative biology and host range of two root herbivores for the biological control of scentless chamomile

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Scentless chamomile, *Tripleurospermum perforatum* (Mérat) Laínz (Asteraceae, Anthemideae), is a weed of disturbed and agricultural land in the prairie provinces of North America. The biology and host range of two root-feeding weevils, *Diplapion confluens* Kirby and *Coryssomerus capucinus* (Beck.), was studied at field sites in the Rhine Valley, in a common garden and in the laboratory from 1993 onwards. The aim was to evaluate their suitability and safety as biological control agents and to detect potential competitive interactions. Both species are univoltine; females started to lay eggs in early spring. *Diplapion confluens* has three, *C. capucinus* five larval instars. Larvae and adults of *D. confluens* are thus smaller, but developed slightly faster. Females of *D. confluens* laid more eggs under laboratory conditions, and usually more individuals were found per plant relative to *C. capucinus*. Larvae of both weevil species were found in the field from mid April until the end of July, when plants set seeds and dried up. Later instars preferentially fed in the vascular cylinder of the shoot base, root crown or root. Whereas *D. confluens* pupated in the plant, and adults emerged the same summer and overwintered in the leaf litter, *C. capucinus* pupated in the soil, and adults emerged the following spring. Although larvae of both species occupy the same temporal and spatial niche and showed similar distribution and attack patterns in the field, no negative or positive interspecific association was detected.

Host-specificity tests were conducted with 43 plant species and cultivars in six tribes of the Asteraceae. Emphasis was placed on plant species recorded as hosts of the two weevils in the literature (e.g. *Anthemis* spp.), on plants closely related to the target weed (27 of the tested plants are in the tribe Anthemideae), on plants of economic importance (e.g. *Matricaria recutita, Chrysanthemum* spp.), as well as on species native to North America (e.g. *Artemisia* spp.). Apart from host range tests under confined conditions, we collected plants growing intermixed with scentless chamomile, we regularly sampled commercial chamomile fields, and we carried out open field tests with augmented numbers of insects. Both herbivores were specific to plant species in the tribe Anthemideae, and scentless chamomile was the preferred host plant in most tests. However, the two weevil species developed to mature larva or to adulthood on several ornamentals (e.g. *Anthemis sancti-johannis*, *Chrysanthemum carinatum*), as well as on one plant species native to North America (e.g. *Tanacetum huronense*) under single-choice or field conditions. The herbal chamomile, *Matricaria recutita*, was only slightly attacked by *Diplapion confluens* under natural field conditions, but when weevils were augmented, *M. recutita* was accepted to the same degree as the control. Although neither of the two species is recorded as a pest of any commercially grown plant species in the tribe Anthemideae, anticipated outbreak densities of the weevils after release may lead to non-target effects. Therefore we decided that both root herbivores are unsuitable for field release in North America.

Minimizing the environmental risks of natural enemy introductions for biological control of greenhouse pests: use of criteria for determining the non-establishment of exotic arthropod predators and parasitoids in the field

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Environmental risks of introducing arthropod natural enemies for biological control of greenhouse pests should be lower than those for classical biological control, a prerequisite for which is the establishment of the natural enemy in the target areas. However, exotic arthropod predators and parasitoids released in greenhouses may escape, establish and attack native non-target hosts or prey. Minimizing these environmental risks is possible by screening the exotic natural enemies before their introduction, based on some criteria for determining their non-establishment, especially in winter. One criterion for natural enemy attributes in relation to climate is to be non-diapausing in the field as they cannot survive winters. Low tolerance for low temperature and developmental threshold temperatures lower than those of hosts may be among such criteria, but to be non-diapausing is a most reliable criterion.

The (theoretical) evolution of agent-target-non-target interactions in biological control

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Most scientists regard the use of agents (or "natural enemies") such as predators, parasites and pathogens for the control of animal, plant and microbial pests to be more of an art than a science. Indeed, in most instances of biological control it is: criteria for the selection, multiplication and release of biological control agents usually have little or no theoretical or experimental backing. The theoretical and empirical foundations for a science of biological control do indeed exist, but that they are often communicated to more applied workers in too technical and indirect ways. I will focus on insect parasitoïds (most often hymenopteran wasps) which are the most often cited success stories in the biological control of insect pests of agriculture. I will discuss a new and exciting perspective on biological control: how the evolution of populations may effect both target and non-target species of biological control efforts. In particular, I will use recent evolutionary theory towards answering the following questions:

1) Under what conditions can rapid evolution occur in biological control systems?
2) Could we mistake evolution for "something else" and *vice versa*?
3) Can an agent evolve to pose a threat for non-target organisms?
4) Could a non-target organism lessen the efficacy of biocontrol via an evolutionary response?
5) What types of system are most vulnerable to non-target effects?

Predicting and assessing non-target impacts of parasitic Hymenoptera attacking *Bemisia* (Homoptera: Aleyrodidae) in the southwestern USA

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Widespread economic damage caused by increasing populations of *Bemisia* in the U.S.A. during the past twelve years led to extensive multi-agency research directed at basic biology and management. Worldwide exploration for effective natural enemies was initiated, followed by evaluations and releases of some of these non-indigenous enemies in the U.S.A. When local surveys and foreign explorations for natural enemies were begun, few of the natural enemy species were adequately characterized taxonomically, and their impact on *Bemisia* populations was not well understood. Existing knowledge of whitefly natural enemies was adequate to recognize certain families and genera of natural enemies closely associated with whitefly, while the host/prey ranges of other taxa were much broader. Project scientists worked closely with taxonomists and molecular biologists to develop identifying characters, and eventually names or markers, for new and nominal natural enemy species. Environmental assessments for *Encarsia* (Hymenoptera: Aphelinidae) and *Eretmocerus* (Hymenoptera: Aphelinidae) were prepared which resulted in release permits for all non-indigenous *Eretmocerus* species reared from *Bemisia.* All species of *Encarsia* reared from *Bemisia* were also permitted for release, although autoparasitic species of *Encarsia* received special scrutiny because males of these species utilize primary whitefly parasites as hosts. An important consideration in this decision was that autoparasitic *Encarsia* species are widespread in many naturally occurring complexes of natural enemies; and there are cases where importation biological control has resulted in improved biological control while creating new natural enemy associations which include autoparasitic *Encarsia.*

In the southwestern U.S.A. where the economic impact of *Bemisia* has been especially severe, pre-release surveys of *Bemisia* and other native whitefly were conducted to identify the native parasite complexes in agricultural, urban and surrounding desert habitats. Thus, when release programs were implemented, native parasites were known and were distinguishable from exotics. Surveys of non-target whitefly have been continuous since 1993; the parasites recovered have been under study and the species are being characterized for publication in order to document the native parasite complexes. Following six years of releases there is convincing evidence that several species of non-indigenous *Eretmocerus* are established in Texas, Arizona and California. As yet, we have no evidence of any of the introduced species reproducing on the non-target whitefly species monitored in the southwestern U.S.

Understanding the prospects for biological control of alien invasive pines (*Pinus* species) in Southern Africa through ecological studies and experimentation in their native habitats in Europe

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Biological control of pines has never been attempted before. In South Africa, *Pinus* species are the basis of an important and lucrative industry (as they are elsewhere), but they are also a substantial threat, as alien invasive plants, to major conservation areas and to the country's meagre water supplies. As a biological control initiative, the project presents an extraordinary juxtaposition of:
i) positive aspects (e.g. an extensive knowledge of the ecology of the target *Pinus* species and of their insect and pathogen associations; the fact that there are no native *Pinus* species in Southern Africa; and the potential to use cone-and seed-destroying insects to avoid conflict of interests);
(ii) negative aspects (e.g. an imperfect knowledge of the details of the host-specificity/biotypes of potential agents and the consequent risk to non-target *Pinus* species; the complexities of pathogen associations; and the expense to growers of protecting seed orchards should biological control be implemented).

In this presentation, we summarise this background and provide details of ecological factors and procedures in South Africa and of field experiments that are in progress in Europe, the native habitat of the target pines. The study will provide a better understanding of the cone- and seed-destroying insects that are under consideration as biological control agents and enable decisions to be made about how the project should proceed.

Indirect effects in the biological control of arthropods with arthropods

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Parasitoids and predators are well known as biological control introductions, but studies on their potential ecological impact and non-target effects are less well advanced than for weed biological control. The spectrum of introduced arthropod agents is changing as a result of a growth in commercial biological control, with predators becoming a more common introduction and some associated changes in host specificity. This paper reviews and analyses known non-target effects of arthropods introduced as biological control agents for other arthropods. It then reports on a new, five-country European initiative, evaluating risks of biological control, and its progress in developing population dynamics models to assist measurement of non-target effects and in direct evaluation of risks from parasitoids and predators introduced classically or augmentatively in Europe.

Non-target effects in biological control - community interactions and the contribution of ecological modelling

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The targets of biological control rarely live alone, but instead are components of often complex webs of interacting non-target species, involved in a multiplicity of complex interactions with other species. These interactions may in turn vary in time, and are often mediated by flows of individuals through space. A useful approach between the baroque complexity of entire communites, and the bare bones of single and pair-wise population dynamics, is provided by close analyses of 'community modules' - small numbers of species (e.g., three to six) linked in a specific structure of interactions. Familiar community modules include exploitative competition for a limiting resource, food chains, shared predation, predation upon competing prey, and intraguild predation. Less familiar modules include systems with a mixture of predation and mutualism, and systems with significant non-trophic interspecific interactions. Most previous studies of modules have emphasized equilibrium states (including bounded oscillations) and have ignored spatial dynamics. This chapter will review this literature, for a selection of these modules, but also extend current perspectives by

1) examining transient dynamics following introductions of control agents (which can lead to likely extinctions of non-target species not apparent from examining equilibrium conditions), and
2) explore module dynamics in a spatial context (e.g., 'spillover' between habitats, and metapopulation dynamics).

The spatial patterning in multispecies interactions is particularly important in gauging the likely long-term evolutionary trajectories of biological control systems. Theoretical models do not provide precise recipes for concrete field situations, but instead help to clarify the range of possible scenarios field workers need to consider in designing control programs.

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The off-target impact of biocontrol on a native Hawaiian stink bug

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Concern over the environmental safety of biological control focuses on its potential impact on non-target species. This debate is especially relevant in Hawaii, where biocontrol is a valuable tool for managing problems with alien species, but where conservation of native species is also vital. We have been studying the specific case of biocontrol of Southern green stink bug (*Nezara viridula*) to assess its impact on the endemic koa bug (*Coleotichus blackburniae*), and to see what lessons can be learned to improve safety of biocontrol in the future.

In the 1960's two species of parasitoids were established in the Hawaiian islands to control a newly arrived agricultural pest, the Southern green stink bug (Davis, 1964). These natural enemies, the egg parasitoid *Trissolcus basalis* and the tachinid fly *Trichopoda pilipes*, have been implicated in the apparent decline of the native koa bug (Howarth, 1991); however our two year study is the first attempt to carefully examine the interaction of these species.

Both parasitoids were found attacking koa bugs in the field on four islands, but life table studies indicate that accidentally introduced natural enemies, including spiders and ants, currently have greater overall impact on koa bug populations. Parasitism by the tachinid was high at some locations, where continued monitoring is recommended. In many areas of Hawaii, the host plants of koa bugs have been displaced, which probably also has contributed to koa bug population decline.

Laboratory tests and historical data (Davis, 1964) suggest that both the tachinid and the egg parasitoid were pre-adapted to successfully locate and utilize koa bugs as hosts. We examined methods for screening these natural enemies, particularly the influence of arena size and complexity. Our case study confirms the relevance of screening non-target hosts and understanding the ecology of natural enemies before their release for biological control.

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Development of host specificity tests for predators as biological control agents: an example for *Clitostethus arcuatus* (Rossi) (Coleoptera: Coccinellidae) on *Bemisia tabaci* Gannadius (Homoptera: Aleyrodidae) complex B-biotype species complex

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The importation and movement within North America of arthropods used for biological control arouses questions and concerns of host range. For the biological control of weeds there is a generally accepted protocol for testing host range. For biological control using insect parasitoids, host range is limited by evolutionary considerations and can be easily assessed. However in the case of predatory arthropods for biological control of other arthropods there is neither an agreed protocol for study nor is host range as limited as in parasitoids.

Clitostethus arcuatus (Rossi) is widely distributed in the Mediterranean and surrounding areas, and is recorded as feeding on whiteflies (more than 10000 whitefly eggs/ individual) (Bathon & Pietrzik, 1986). Their potential for use in the biocontrol of pest whiteflies is of great interest.

We report here on tests conducted using *C. arcuatus* during the development of a protocol for assessing host range in predators, using adult insects. The dimensions of the recorded prey of *C. arcuatus* (itself 5mm long) range from 0.1mm (*Tetranychus urticae* eggs) to 2.25mm, (adult *Myzus persicae*). Small test arenas were therefore thought to be appropriate.

The specificity of *C. arcuatus* was tested using adults of 2 whitefly species *Bemisia tabaci* Gannadius, *Aleyrodes proletella* L. and the nymphs of an aphid of the same size *Brevicoryne brassicae* (L.), offered in 2 forms of arena; an aerated petri dish arena containing a cabbage leaf disc onto which the prey insects and the predator were introduced; a rooted single leaf of cabbage enclosed in a cellophane bag. The first system, though easy, to use was not appropriate for adult whiteflies as condensation and static electricity caused high mortality. The second system which allowed air movement through the membrane and was not subject to static charge eliminated mortality of whitefly adults due to condensation and static.

The results of the tests in the petri dishes were subjected to a two way factorial analysis of variance, comparing treatment effects using adult *C. arcuatus* (blank check, male, female) as one factor and prey species (*B. tabaci*, *A. proletella* and *B. brassicae*) as the other factor.

Female *C. arcuatus* reduced the number of *Bemisia* eggs more than did males. Would testing females only be appropriate or must one test both sexes, therefore doubling the work? Significantly more aphids survived in tests than did either species of whitefly. No significant difference in survivorship was observed between the 2 species of whiteflies.

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Natural host specificity assessment of European parasitoids for classical biological control of the cabbage seedpod weevil, *Ceutorhynchus assimilis*, in North America: evaluation of potential non-target risks

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The cabbage seedpod weevil, *Ceutorhynchus assimilis* Paykull (Coleoptera: Curculionidae), is a serious exotic pest of canola and rapeseed (*Brassica napus* L. and *Brassica rapae* L., respectively) throughout much of North America. As the future availability of insecticides is uncertain, there is a critical need to more effectively utilize biological control in the future. Several hymenopteran parasitoids of cabbage seedpod weevil are known from Europe. Despite many proven benefits, classical biological control has recently come under scrutiny by conservationists and environmentalists because of the concern that imported natural enemies may adversely affect native fauna, especially rare and endangered species. For a long time, there was little concern about the fate of alternative hosts of the parasitoids of arthropod pests unless obviously valuable species were at risk. As a result of the earlier lack of concern for non-target arthropods, host specificity screening of arthropod natural enemies was non-existent or perfunctory until the last decade. Therefore recent theoretical papers are suggesting procedures to evaluate the impact of parasitoids of arthropod pests on non-target hosts (*i.e.* Sands, 1997, van Driesche & Hoddle, 1997, Hopper, 1998).

The research of this ongoing case study concentrates on assessing the host specificity of European parasitoids of *C. assimilis* and to evaluate the potential risks to on-target Ceutorhynchinae host species in North America. The host specificity of European parasitoids is presently studied qualitatively in open fields in cultivated and non-cultivated habitats. Results of this natural host specificity study will also contribute to understanding whether European parasitoids, released for the biological control of cabbage seedpod weevil, will adversely affect ongoing weed projects that use European species of Ceutorhynchinae as classical weed biological control agents in North America.

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Evaluation of the safety of biological control agents for introduced marine pests

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Exotic marine organisms are being introduced world-wide with increasing frequency and severity of impacts. Despite our best efforts to eliminate the vectors of marine introductions, we will not be able to prevent them all. Although prevention will always be a very desirable option, it cannot supplant the need to mitigate pests that are already here, or are yet to come. However, until very recently, there has been no effort to control those introduced marine pests that have become established. This defeatism is unique to the pest control in the marine environment. Based on models from agricultural insect pests, weed control, and the impact of infectious diseases on fisheries, we have developed a biological control approach to reduce the impact of introduced marine pests (Lafferty & Kuris, 1996). Host-specific natural enemies such as parasitic castrators, have been proposed as potentially effective natural enemies and have parasitoid-like attributes. We are evaluating their safety and efficacy against the alien european green crab, *Carcinus maenas* (Linn.) (Portunidae), in California. Safety is of critical concern for management agencies and the public. The design and evaluation of host-specific safety tests will be discussed and compared with safety of biocontrol agents in terrestrial and freshwater environments.

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Introduction of an exotic egg parasitoid - a potential risk for a native tachinid fly?

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The introduction of exotic generalist egg parasitoids may have direct negative effects on populations of non-target insect species and indirectly on native competitors. *Lydella thompsoni* Hert. (Dipt.: Tachinidae) is a native larval parasitoid of the European Corn Borer, *Ostrinia nubilalis* Hb. Parasitism rates can reach 50% or even more in Southern Switzerland. The spring generation of the tachinid develops on hosts in natural habitats, while *O. nubilalis* is the main host for the two subsequent generations. In order to control *O. nubilalis*, inundative mass releases of the exotic egg parasitoid *Trichogramma brassicae* Bezd. (Hym.: Trichogrammatidae) are carried out annually. These releases coincide with the oviposition period of known spring hosts of *L. thompsoni*. Introduced *T. brassicae* disperse from maize fields into natural habitats in the surroundings, where they might attack eggs of non-target hosts, such as *Archanara geminipuncta* Haw. (Lep.: Noctuidae) and *Chilo phragmitellus* Hb. (of *L. thompsoni*). The present case study attempts to determine whether *T. brassicae* competes with *L. thompsoni* for non-target hosts and whether, as a consequence, the mass releases of the egg parasitoid negatively affect the population density of the native tachinid.

A. geminipuncta was found to be the most abundant spring hosts of *L. thompsoni* in Southern Switzerland. Parasitism rates were between 0 and 43%. In laboratory studies, where eggs of *A. geminipuncta* (Lepidoptera, Pyralidae) on common reed (*Phragmites australis* Trin.), which are known as spring hosts were offered to *T. brassicae*, only one female *T. brassicae* was able to successfully parasitize a single egg of this noctuid moth (n=20). In addition, 2385 eggs of *A. geminipuncta* were exposed in a common reed habitat and checked for parasitism by *T. brassicae*, which were released in adjacent maize fields (900.000 females/ha). These field studies revealed no parasitism by *T. brassicae*. Another potential host, *Chilo phragmitellus* is less abundant than *A. geminipuncta* within the study area, and so far has not been found to be parasitized by *L. thompsoni*. A preliminary lab experiment, where single *T. brassicae* females were provided with *C. phragmitellus* egg masses, showed that 5 of 6 moth egg batches and 63% of the eggs (n=59) were successfully parasitized. Results from a field experiment will be presented and discussed as well.

Our data demonstrate that *A. geminipuncta*, the most important spring host for *L. thompsoni*, is not at risk by introduced *T. brassicae*. In contrast, *C. phragmitellus* egg masses are attractive to *T. brassicae* and mass releases of this exotic egg parasitoid may negatively affect populations of the non-target host. As the native tachinid fly *L. thompsoni* shows a strong affinity to *A. geminipuncta*, whose eggs are not parasitised by *T. brassicae*, interspecific competition between exotic *T. brassicae* and native *L. thompsoni* seems to be rather unlikely (in Southern Switzerland).

The outcome of the introduction of a pathogen for the biological control of pest grasshoppers (Orthoptera: Acridoidea) in Argentina

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Between 1978 and 1982, the protozoan pathogen *Nosema locustae* was introduced from North America into central (Pampas) and southwestern (Patagonia) Argentina for the control of pest grasshoppers. Seven introduction localities were in the Pampas and two in Patagonia. The short-term impact (control within seasons of application) of the introductions will remain unknown because reports were not produced and data on infectivity and density reductions are not available. Similarly, no efforts were made to evaluate the long-term outcome of the introductions, and the fate of *N. locustae* also remained unknown for years. In 1991, the pathogen was re-found parasitizing three species of grasshoppers (Lange, 1992), and since then monitoring activities for its presence were initiated (Lange & de Wysiecki, 1996). Up to now, establishment of the agent in grasshopper communities was observed in two well-defined areas: Gualjaina in Patagonia, and an area in the western Pampas surrounding three of the application sites. Infections were diagnosed in 14 species of grasshoppers. Maximum geographic dispersion recorded was 160 km. At present, 181 species of grasshoppers are known for Argentina, and all are native (*i.e.* not introduced). Between 8 and 16 species might be present in a season in the areas where the introductions were made. Some are of clear economic importance, qualifying as targets in control programs. Eleven out of the 14 species have been mentioned at least once as causing damage. However, the other three plus others that might be suffering infections but were not detected yet, should probably be considered as non-targets. Lockwood (1993) raised concerns about the use of exotic microorganisms for the control of native pests, an approach that he named Neoclassical Biological Control. He believes that, among other negative impacts, such a strategy could lead to extinction of non-targets. Carruthers & Onsager (1993) have a different perspective, and disagree. Although costs/benefits evaluation of such an approach might be difficult (even impossible) due to a number of factors (notably, the drastic environmental changes), the establishment of *N. locustae* in Argentina provides an opportunity for addressing some of the issues at stake.

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Selecting hosts resistant to parasitism as a potential side-effect of biological control: the case of *Aphis gossypii* Glover

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Selecting hosts resistant to parasitism is one possible non-target effect of biological control. However, as pointed out by Holt & Hochberg (1997), no example of an increase in insect host resistance to parasitoid can be undoubtedly attributed to released parasitoids. The question remains whether this potential selective pressure represents a real risk or not in biological control.

Parasitism failure due to behavioural or physiological host defence has frequently been described. For aphids such as *Aphis gossypii* Glover which colonise greenhouses, the situation is particular: they are strongly aggregated; one clone often colonises one greenhouse (Fuller *et al.*, in press); they produce winged individuals at the end of the crop period, which constitute the propagules participating to genetic mixing before new area colonisation; when stung by parasitoid species such as *Lysiphlebus testaceipes* Cresson or *Aphidius colemani* Viereck, they are still partially able to produce offsprings. For these reasons, selective pressures exerted by the parasitism have to be evaluated not only at the individual level but also at the scale of the clone distribution area, eventually after several generations.

Using these particular host-parasitoid associations, we observed that parasitism failure may occur from: (1) an increase in host mortality, during the days following the parasitisation; (2) a host escape from mummification (host death and nymphal moult of the parasitoid), with a modified fecundity, and (3) a parasitoid death inside the mummy, without adult emergence. We developed a delayed differential equation system to model aphid-parasitoid dynamics in greenhouses, including residual fecundity of stung hosts and the three kinds of parasitism failure observed. Laboratory experiments were done to evaluate the model parameters.

In the experimental conditions used, the rates of parasitism failure were 29% and 18% when hosts were stung by *L. testaceipes* and *A. colemani* respectively. Most of the parasitism failure was due to stung hosts escaping from mummification. These individuals showed a longevity similar to unparasitised controls. Their fecundity was not modified when stung by *L. testaceipes* but was reduced when stung by *A. colemani.*

Individual and kin host advantages are discussed according to the different kinds of parasitism failure and to the results of the model simulations.

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Evaluation of impact in weed biological control

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Most weed biological control systems are fairly simple and yet not readily predictable. Furthermore, although rigorous specificity testing means that an agent can be expected to be fairly closely coupled to the weed, the addition of another agent, plant competition, or an alternative host into the system, will produce even less predictable dynamics. In addition, the measurement of agent impact on the weed is often difficult for practical and statistical reasons. As a consequence, quantitative demonstrations of impact on weed population dynamics are comparatively few. The difficulties are magnified if we attempt to measure impact on non-target species, particularly if these are rare. Other higher order interactions, such as competition between the introduced biocontrol agent and related native species, would be yet more problematic. We present some possible solutions to these problems, and look at the prospects for measuring off-target impacts of biocontrol agents in a way that identifies impacts at a population level. In particular, we explore the application of the criteria used by the IUCN (The World Conservation Union) for identifying critically endangered, endangered and vulnerable species, to quantifying the degree of risk imposed on non-target species from biocontrol. We also look at the design methodologies employed in environmental impact assessment, and examine their application to biocontrol impact.

Do exotic parasitoids introduced for whitefly control endanger our environment?

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In the history of biocontrol, aphelinid parasitoids have become key components of reliable and economic pest management and sustainable agriculture. Exotic species (e.g. *Encarsia*, *Eretmocerus* species) have been widely introduced and released for the control of exotic whitefly and scale pests in classical programmes and currently billions are released seasonally or inundatively into protected crops. In spite of their wide usage, relatively little is known about the potential ecological effects of these releases. Here we present an overview of the direct and indirect ecological effects of these releases in various agricultural and climatic settings and ecosystems, including the implications of their use in biological control programmes. Particular reference is made to the effects of the competitive outcome between primary parasitoids and heteronomous hyperparasitoids (co-existence, displacement effects), between native and exotic parasitoid species, their effects on native and exotic host species, outdoor survival and dispersal abilities, and the potential impact for the ecosystems they invade. First results are presented of our surveys of native European whitefly species and the potential non-target impacts of introduced species on these species.

Magnitude and mechanisms underlying indirect non-target effects of *Rhinocyllus conicus* on native inflorescence insects

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Assessment of the magnitude and mechanisms of indirect non-target effects is critical for both basic understanding of interactions and for practical decisions, such as risk assessment associated with classical biological control. *Rhinocyllus conicus*, a flowerhead weevil imported into North America for the biological control of Eurasian thistles in the genus *Carduus*, is now having both direct and indirect non-target effects on native *Cirsium* thistles and their adapted insects. We present both our protocol and the initial results of post-release evaluation of the indirect consequences of *R. conicus* feeding on inflorescence-feeding insects of *Cirsium canescens*, a characteristic native thistle of the upper Great Plains, USA. By 1996, observational evidence suggested that the numbers of a native insect, the tephritid *Paracantha culta,* may have declined as numbers of the weevil have increased. Using an experimental manipulation of oviposition by *R. conicus*, we have quantified the interaction and demonstrated that the effect is not just correlative. The underlying mechanism is complex, combining altered fly behavior in the presence of *R. conicus* eggs, preemptive resource consumption of the smaller flowerheads by *R. conicus*, and weevil interference with fly feeding position in the larger flowerheads. These data quantify the indirect effects associated with alteration of guild structure, and ways in which such interactions may be analyzed for other on-going and future biocontrol projects are suggested.

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Transient impacts in biocontrol: factors determining minimum non-target densities

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Situations where there are non-targets that are threatened or of some interest, and which are similar (in the same trophic level) to the intended target, offer several possibilities for the ecological nature of a non-target impact in classical biocontrol. Firstly, there is the chance that an agent may establish on the non-target, even in habitats where the target is absent, with perhaps severe population consequences. Where such establishment is not likely (due to densities of the non-target host/prey, suitability or conversion efficiency of the host/prey, or searching efficiency), there are two main potential population consequences. Sustained densities of the agent on the target host or prey, where some of these flow over into the habitat of the non-target, can lead to a sustained long-term suppression. This is more or less synonymous with the effects of apparent competition. However, additional, more severe effects can be anticipated soon after the onset of biocontrol, as the introduction is likely to be followed by large peaks of agent densities, implying transient minimums of non-targets. It is these transient effects which we examine here, as these are most likely to lead to (local) non-target extinction, even where relative searching efficiency, or preference, for the non-target is very low. In simple host-parasitoid models we examine the factors which may influence the size of the non-target impact. This has revealed some important, and hopefully quite general, messages about when non-target impacts are likely.

It seems that the main determinants of the size of a non-target impact are the density of the host which is to be controlled, the conversion efficiency of these hosts into parasitoids and the nature of the process by which non-target hosts are found (such as the searching efficiency on the non-target and the proportion of hosts which make it to the non-target habitat). As absolute searching efficiency on the non-target is an overriding factor, agents can have relatively low attack rates on the non-target (as compared to the target) yet the non-targets can suffer quite a large transient impact. Some details of the population dynamics of the non-target influence the minimum non-target density, for example, realised fecundity (or population growth rate) and nature of natural density dependence (e.g. scramble versus contest competition), but do so in a relatively subtle way. Depending on the nature of the local dynamics of the target-agent interaction, and, of course, whether host populations are sympatric or not, either the searching efficiency for the non-target assessed under choice (target host present) or non-choice (target host absent) conditions is most appropriate for predicting the severity of non-target effects. As this may be quite difficult to predict in advance, in this context non-choice searching efficiencies may give indicators of impact on the side of caution.

Practical use of systematic and ecological analyses to determine non-target species for host-range testing of entomophagous biological control agents

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The effect of introduced agents on hosts other than the target species is an issue of increasing importance for biological control practitioners. While this aspect has been a component of the evaluation process for phtyophagous agents for some time, non-target host evaluation of entomophagous biological control agents is a relatively recent development and there is a need to develop methodologies tailored to entomophagous agents. Although assessment of the host specificity of phytophagous biological control agents has been based primarily on the phylogenetic methods developed by Wapshere (1974), these methods may not provide a good model for similar studies with entomophagous agents. Incomplete taxonomic information impedes the ability to select appropriate phylogenetic non-target species for testing. Additionally, ecological factors influence host distribution and hence parasitoid specificity.

Study of the plant bug genus *Lygus* and the nymphal parasitoid genus *Peristenus* illustrate some of the issues that must be dealt with when evaluating entomophagous biological control agents (Kuhlmann *et al.*, 1998). These include the need to develop a phylogeny for the Miridae that reflects ecological as well as taxonomic attributes; review the taxonomy of the parasitoid genera *Peristenus* and *Leiophron* to clarify species complexes; and determine the habitat specificity of potential non-target host mirids.

For entomophagous biological control agent host specificity testing it is recommended that: 1) local populations of candidate entomophagous biological control agents be designated as the test unit and 2) candidate non-target species be selected on the basis of similarity of habitat to that of the target species.

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Establishment and impact of three biological control agents on purple loosestrife, *Lythrum salicaria* L. (Lythraceae), and non-target plants in Virginia

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Three sites in Virginia were selected for the release of exotic agents for the biological control of purple loosestrife. Density (% cover) of plant species were recorded before and after release using regularly spaced m^2 plots. The first site was in Coeburn, where an infestation of purple loosestrife grows along a small stream through the town for approximately 1 km long and 3 m wide on both sides of the stream. *Galerucella calmariensis* (600 adults) (Coleoptera: Chrysomelidae) and *G. pusilla* (1,100) were released during the summers of 1992 and 1993. Between 1992 and 1996, a total of 5,300 eggs and 103 adult *Hylobius transversovittatus* (Coleoptera: Curculionidae) were also released. From 1997 to 1999 non-target plant species averaged 4.2 per plot and 26.3 species for the 175-m-long transect. No other species in the Lythraceae family were found. Non-target species were not affected by different levels of purple loosestrife infestation (Kruskal Wallis non-parametric test, $P > 0.05$).

In August 1996 this streambed was channelized and the banks reseeded primarily with *Poa pratensis*. Approximately 0.5 m of soil along the streambed and streambank were removed. The *Galerucella* beetles were not noticeably affected by the channelization. This may indicate that these beetles found habitats for diapause above the flood plain. Mean egg densities of the two beetles steadily increased from 0.06 to 24.1 eggs/stem/m^2 with a mean of 2.3 eggs per egg mass. The *Galerucella* beetles covered an area of 92 to 4,400 m^2 between 1993 and 1999. The ratio of the two *Galerucella* spp. varied with *G. calmariensis* constituting 53, 98, 32, 30, 62, 65 and 32% of the beetle population, respectively for each of the years from 1993 to 1999. Although the mean number of stems did not change (8.2 stems per m^2 from 1997 to 1999), the percent cover of purple loosestrife declined from 16.6% in 1997 to 11.4% in 1999. Feeding impact was apparent on the inflorescence. Length of the inflorescence decreased from 116 cm in plots with no *Galerucella* spp. to a mean of 9.5 cm with a mean of 24.1 eggs per stem. This was a 92% reduction in flowering.

The second site was in Goshen, Virginia and is an undisturbed wetland of approximately 6,600 m^2. 32 non-target plant species were recorded from 41 m^2 plots along a 110 m long transect with a mean of 3.0 non-target species per plot. Purple loosestrife cover per m^2 was 67%. No other species in the Lythraceae family were found at this site. Purple loosestrife significantly reduced the number of non-target plant species (Kruskal Wallis non-parametric test, $P < 0.05$). 3,000 *Galerucella* spp. adults were released from 1994 to 1996 and 5,000 in 1997. Both species have been recovered since 1995 in low densities. The two *Galerucella* spp. in 1999 covered 3,290 m^2 of this site with a mean of 0.4 eggs per stem. No significant defoliation or reduction in inflorescent length has occurred. Deer browsing during the spring is high at this site and may be inhibiting the density of *Galerucella* spp. eggs and larvae. Ten females, 9 males and 2,000 eggs of *H. transversovittatus* were released at this site between 1994 and 1996. In November 1998, 5 of 137 roots and in March 1999, 2 of 120 roots had at least 1 *H. transversovittatus* larvae, indicating establishment.

At the third site, approximately 700 adult *G. calmariensis* were released at Beaver Dam Falls, Virginia, in 1994 and 1995. In 1999, the population had increased significantly to defoliate purple loosestrife and prevent flowering on 80% of the plants.

Predicting non-target effects of weed biocontrol agents: lessons from a case study of *Lema cyanella* and thistles (*Cirsium* spp.)

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Canada thistle, *Cirsium arvense* (L.) Scop. (Asteraceae), a perennial European thistle with creeping roots, is a major weed problem across Canada and the northern USA. In 1983, the leaf-feeding beetle *Lema cyanella* (L.) (Coleoptera: Chrysomelidae) was approved for release in Canada as a biological control agent against *C. arvense*. Approval was based on field records from the native range suggesting that the insect was specific to *C. arvense* (Zwölfer, 1965; Zwölfer & Pattullo, 1970), choice and no-choice feeding tests in petri dishes, and field cage tests (Peschken & Johnson, 1979, Peschken, 1984). Feeding, oviposition, and development occurred on some native North American *Cirsium* species in these tests. However, it was argued that, according to the resource concentration hypothesis (Root, 1973), rare or scattered non-target *Cirsium* species would be less susceptible to attack by *L. cyanella* than the abundant target.

After the first field releases in 1994, we tested the predictions made from pre-release testing by conducting further host-preference experiments in open field plots and large field cages, using several native North American *Cirsium* species. These showed that *L. cyanella* discriminates strongly among *Cirsium* species, but that it does present a significant risk of damage to some native species in the genus. One native non-target *Cirsium* species was heavily attacked in open-field host preference tests, even when it occurred as single plants within large stands of *C. arvense.* The resource concentration hypothesis is thus not a reliable basis for predicting non-target effects. Field cage tests and choice tests with cut leaves were fairly good predictors of préference in the field. Another European thistle, *Cirsium vulgare* (Savi) Ten., was heavily attacked, although *L. cyanella* has rarely been found on it in Europe. Field host records from the native range therefore need to be interpreted with caution. Especially when the insect is rare, the scarcity or absence of records from a plant species does not necessarily prove that the plant is not an acceptable host. On the basis of these studies, releases of *L. cyanella* have been discontinued.

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How reliable is host specificity as a measure of safety in weed biocontrol?

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The safe and effective use of biocontrol requires assessing the control organism's ability to harm non-target organisms, survive, reproduce, disperse, and evolve. Here, I examine host specificity of biocontrol agents, which is one of the primary criteria that scientists and regulators use to evaluate and rank the risks that biocontrol agents pose for non-target organisms.

The scope of host specificity tests is often too narrow to predict which organisms are likely to be attacked in the release environment. Host specificity tests typically measure the potential of the control organism to complete its life cycle on the target organism and also on the non-target organisms it consumes. In traditional host tests, vulnerability is equated with suitability for larval development, but this assumption can be an unreliable basis for predicting host use in the field. The situation arises because host selection is a hierarchical sequence of opportunities and constraints, of which the suitability for development is just one component. Thus, screening tests of potential control organisms and their hosts must be expanded to include investigation of how the probability and intensity of their interaction depends on phylogenetic, genetic, physiological, behavioral, and ecological constraints. In well-designed tests, the boundaries of the physiological host range measured in the lab may be unacceptably broad, but the estimate of the host range grows progressively narrower (and possibly more acceptable) as behavioral and ecological constraints are considered. Once the probability and intensity of host use are known, the consequences for the host population must be estimated.

Even the best host specificity tests may prove to be insufficient to estimate the probability and severity of target and non-target effects. This is because a control organism may harm a non-target organism in a multitude of ways-from a direct feeding relationship that arises when the control organism consumes a non-target organism, to direct interference competition, to indirect interactions that can arise when the control organism and the non-target organism interact via intermediate species such as a shared natural enemy or a shared host. Additional tests need to be carried out. The potential to survive and reproduce requires assessing the control organism's rate of increase to predict the conditions likely to generate outbreaks of the control organism. The potential to disperse requires assessing of the control organism's movement, whether by active or passive transport, to estimate the probability of its moving a given distance in a given amount of time. The potential of the control organism to evolve and adapt to new hosts and environmental conditions requires examining the organism's evolutionary history as well as the interplay of genetic variation, natural selection, and ecological opportunity for organism interactions. For organisms with the potential to harm other organisms, the risks become greater (and harder to predict) as the control organism's ability to survive, reproduce, disperse, and evolve increases.

Despite its shortcomings, host specificity continues to be a reliable concept for assessing safety in biocontrol. Of 8 reported cases of direct harm to non-target species by weed-control organisms, all could have been avoided if host specificity were enforced. As an added measure of safety, the likelihood of indirect effects also decreases as host range becomes narrower. The most important uncertainties that remain have to do with evolutionary stability of the host range.

Field experiments and surveys in the weeds' native range to solve contradictory results of quarantine host-specificity studies: *Solanum* weeds case study

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Determination of the host range of potential candidates (insects) for weed control under experimental conditions often leads to ambiguous results. The artificial nature of the quarantine testing process may overestimate the range of plants that the agents actually feed on in nature. Host-specificity studies (non-choice and multiple choice feeding and oviposition tests) with phytophagous insects introduced into quarantine in South Africa and in the USA for biocontrol of *Solanum* weeds have produced unexpected results. Complete development on eggplant (*Solanum melongena*), an economically important non-target solanaceous crop, has been obtained in quarantine tests with chrysomelid beetles (*Gratiana spadicea, Leptinotarsa texana, Leptinotarsa defecta*, and *Metriona elatior* in South Africa; *Gratiana boliviana* and *Metriona elatior* in Florida, USA) for *Solanum* weeds (*Solanum viarum, Solanum elaeagnifolium, Solanum sisymbrifolium, Solanum mauritianum*). Therefore, we are now placing more emphasis on open-field experiments and surveys in the weeds' native region to corroborate quarantine findings.

Field surveys, conducted from June 1997 to May 1999, of insects attacking non-pesticide treated *S. melongena* plants in Argentina (8 fields), Brazil (11 fields), and in Uruguay (1 field) indicated that *S. melongena* is not a host plant of *G. spadicea, G. boliviana*, and *M. elatior*. Open-field experiments with *S. viarum* and *S. melongena* in Brazil and Argentina also confirmed these beetles do not attack *S. melongena* under natural conditions. Field surveys and experiments in the country of origin are now routinely included in the screening process, especially in situations of normal development on non-natural hosts in quarantine conditions.

The information that has been obtained from field experiments and surveys in the weeds' native range is allowing a more realistic risk assessment of potential biocontrol agents that otherwise would have resulted in their rejection.

When insect biocontrol interferes with weed biocontrol: selection pressures leading to host shift in a parasitoid of Mediterranean fruit fly

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The braconid parasitoid *Diachasmimorpha tryoni* was imported to Hawaii early in the century to control medfly, *Ceratitis capitata*. It became established on all major islands, and high levels of parasitism were recorded. Subsequent invasion of the islands by Oriental fruit fly competitively displaced medfly from most low elevation sites. Also, imported parasitoids of Oriental fruit fly were superior intrinsic competitors of *D. tryoni* within medfly larvae.

The tephritid *Eutreta xanthochaeta* (lantana gall fly) was introduced to Hawaii for biological control of the weed *Lantana camara*. Host acceptance and host suitability tests proved the susceptibility of *E. xanthochaeta* to parasitism by *D. tryoni*. In recent years, it has become increasingly difficult to find *D. tryoni* in field-collected medflies, while parasitism rates of up to 28% have been recorded in lantana gall fly populations.

Lantana gall flies are larger than Mediterranean fruit flies. *D. tryoni* reared from lantana gall fly larvae are thus larger, more fecund, and presumably more fit than those reared from medfly. Reduced competition from other parasitoids and the greater availability of larval nutrients may be favoring a host shift of *D. tryoni* from medfly to lantana gall fly. The consequences of this for both insect and weed biological control are discussed.

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Non-target impact of exotic natural enemies released on *Maconellicoccus hirsutus* Green in St. Kitts, West Indies

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The exotic natural enemies *Anagyrus kamali* Moursi from China and Hawaii plus *Gyranusoidea indica* Shafee, Alam and Agarwal from Egypt were released on *Maconellicoccus hirsutus* Green, the pink hibiscus mealybug, in St. Kitts, West Indies. These releases occurred from August of 1996 through April of 1998. This mealybug attacks over 200 host plant species and did significant economic damage to numerous ornamentals such as hibiscus at residential properties and hotels plus threatened the agricultural community in St. Kitts. A classical biological control program was implemented in St. Kitts, and was successful in reducing the pest population density by an average of 94% on hibiscus, which was used as the standard host plant for this study. Surveys were conducted one year after the termination of the program during two seasonal periods (March and July of 1999) to determine if these exotic parasitoids were also attacking non-targeted mealybugs.

A total of 10 mealybug species were sampled from around the island of St. Kitts. These species included: *Dysmicoccus brevipes* (Cockerell), *Ferrisia virgata* (Cockerell), *Hypogeococcus pungens* Granara de Willink, *Nipaecoccus* sp., *Paracoccus marginatus* Williams and Granara de Willink, *Phenacoccus madierensis* Green, *Plannococcus minor* (Maskell), *Pseudococcus jackbeardsleyi* (Gimpel & Miller), *Pseudococcus longispinis* (Targioni Tozzetti), and *Puto barberi* (Cockerell). Individual mealybugs of each species were encapsulated in gelatin capsules and held for 30 days to determine if parasitization had occurred. Each individual mealybug parasitized was identified in addition to the parasites. Neither *A. kamali* or *G. indica* were found parasitizing these non-targeted species of mealybugs during these two sampling periods. As a result of these findings, it is concluded that these two exotic species released in St. Kitts had no direct impact on non-targeted mealybug species sampled.

Biological control in Africa and its possible effect on biodiversity

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Biological control efforts in tropical Africa have been most often directed at invading exotic species and are conducted in rapidly changing landscapes, in which the flora, fauna, and ecological interactions are imperfectly known. Faced with major threats to food production and ecosystem destruction, biological control practitioners have been obliged to take a pragmatic approach to minimize the risks of undesirable ecological effects. Workshops convened under the auspices of the Inter-African Phytosanitary Council and FAO have brought together stake-holders and international agencies. Procedures required as preconditions for the importation of biological control agents have usually involved third country quarantine and host specificity tests, which - following the FAO code of conduct - have become more rigorous in recent years. Thus far, extensive pre-release testing of candidate control agents for the ability to attack native relatives of target species has not usually been required. Post-release documentation of impact has often included detailed ecological studies, but these have focused largely on agricultural habitats. The procedures followed and insights gained in respect to indirect effects are discussed in light of classical biological control campaigns involving parasitoids and predators against exotic Homoptera, tetranychid mites, lepidopterous stemborers, thrips, the larger grain borer, and floating water weeds; endophytic fungi against nematodes; and fungal and protozoan pathogens against grasshoppers and other pests.

Insect herbivory may not reduce growth of *Centaurea maculosa* nor reduce its competitive effects on neighbors

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Spotted knapweed, *Centaurea maculosa* Lamarck (Asteraceae), is an invasive plant in North America which is native to Europe and western Asia. Numerous insects have been introduced from Europe to control *C. maculosa*, but it continues to spread (Muller-Scharer & Schroeder, 1993). The primary assumption of biocontrol use is that herbivory will decrease the competitive effect of targeted weeds on native species, but few studies have examined these indirect relationships. We investigated the effects of insect herbivory on *C. maculosa's* competitive effects on two native bunchgrass species in the Rocky Mountains.

In a field experiment, we applied the root borer, *Agapeta zoegana* L. (Lepidoptera: Cochylidae), to *C. maculosa* which were planted with a *Festuca idahoensis*. We found that the reproductive output of *F. idahoensis* decreased when neighboring *C. maculosa* were damaged by *A. zoegana*. In a greenhouse experiment, we subjected *C. maculosa* to herbivory by the cabbage looper moth, *Trichoplusia ni* Hübner (Noctuidae: Plusiinae). We found that damaged *C. maculosa* individuals had stronger negative effects on the root biomass of *F. idahoensis* than undamaged individuals. In neither experiment was *C. maculosa's* biomass significantly reduced by herbivory (Callaway *et al.*, 1999).

In a third experiment, we subjected *C. maculosa* to *T. ni* herbivory ranging from 0 to 90%. The target *C. maculosa* was either planted by itself or with a conspecific, *F. idahoensis*, or *Festuca scabrella*. Our results showed that *C. maculosa* has a different growth response to herbivory depending on the neighboring plant. When *C. maculosa* was paired with either another conspecific, *F. idahoensis*, or *F. scabrella*, the effect of herbivory was significantly less than when *C. maculosa* was grown alone. However, neighbor species that were not exposed to herbivory did not show significant growth responses when the target *C. maculosa* was damaged.

Our results suggest that the indirect effects of herbivory on *C. maculosa* are complex, possibly stimulating compensatory growth and stronger competitive effects on native plants. We hypothesize that mycorrhizal integration of individuals allows damaged *C. maculosa* to increase its growth rates after herbivory.

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The importance of prior experience and population source in the determination of host range

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We have investigated the importance of prior experience and population source on the performance and host preference of two insect herbivores of aquatic plants: the milfoil weevil, *Euhrychiopsis lecontei* Dietz (Curculionidae) and the waterlily leaf beetle, *Galerucella nymphaeae* L. (Chrysomelidae). The milfoil weevil, endemic to North America, is a specialist on watermilfoils (*Myriophyllum* spp.; Haloragaceae) and a control agent of the exotic Eurasian watermilfoil (*M. spicatum* L.). Weevils reared on the native host show equal preference for native (*M. sibiricum* Kom.) and exotic (*M. spicatum*) watermilfoils, however, when weevils are reared on the exotic *M. spicatum* they strongly prefer it over native watermilfoils. Furthermore, the weevil performs better on the newly acquired, exotic host. This preference is induced in adults and does not appear to result from selection, although significant genetic variation in host plant preference was found in a population reared on the exotic for over 30 yrs (Solarz, 1998). The waterlily leaf beetle is a congener of two *Galerucella* species being used for classical biological control of purple loosestrife (*Lythrum salicaria* L.; Lythraceae); *G. nymphaeae* will also feed on *L. salicaria* (Cronin, 1997). Comparison of host preference and performance among populations of the waterlily leaf beetle revealed significant differences in host preference and performance among populations, even when tested on the same plants (Cronin *et al.*, in press).

Furthermore, preference of beetles from one lake varied between years, as did the host they used in the lake. In a given year, each population showed a relatively restricted host range, but across populations and years the host range was much broader. These results indicate that prior experience and population source are important, but often overlooked, considerations in the determination of host range of herbivorous insects.

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Evaluation of ecological risks by using exotic polyphagous predators for biological control.

Laboratory assessment of inter- and intra-specific predation between the exotic *Harmonia axyridis* (Pallas) and the native species *Propylaea 14-punctata* (L.) and *Adonia variegata* (Goeze) (Coleoptera: Coccinellidae)

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The general aim of this study is to evaluate the impact of releases of exotic generalist predators on related indigenous predator and prey species. Laboratory experiments were carried out concerning cannibalism and interspecific predation of eggs by adult females and IV instar larvae of an exotic ladybird and two native ones. The following behavior was studied:

- cannibalism in the exotic *Harmonia axyridis* (Pallas) and the native species *Propylaea 14-punctata* (L.) and *Adonia variegata* (Goeze);
- interspecific predation of the exotic and native species (i.e. *H. axyridis* vs. *P. 14-punctata* eggs; *H. axyridis* vs. *A. variegata* eggs) and vice-versa.

Larvae of coccinellids were reared with *Ephestia kuehniella* eggs; adults were fed with *E. kuehniella* eggs and aphids (*Aphis gossypii* Glover and *Myzus persicae* Sulzer).
All experiments were conducted in glass petri dishes (Ø=12cm) at 25±1°C, RH=70±10%, with constant lighting. Each experiment was replicated 30 times. The predators used in the tests were fed for 24 hours with aphids, then starved for the next 24 hours; a specimen was then put into the petri-dish with 20 Coccinellidae eggs and, eventually, 40 aphids. After 1 hour, the remaining amounts of Coccinellidae eggs and live aphids were counted.

The preliminary results indicated no significant differences between interspecific egg predation by *H. axyridis* and cannibalism by native species done from both adult and larval stage. Data showed also an higher rate of aphid predation for *H. axyridis*, in comparison to the native species. These experiments showed a strong propensity of *H. axyridis* to cannibalise eggs, for both larvae and adult stage. Moreover cannibalism exhibited by *H. axyridis* was higher than interspecific predation exhibited by native species, in both larva and adult stage.

In conclusion our preliminary results seemed to demonstrate that the exotic species (*H. axyridis*) did not show predation on native species higher than their cannibalism, for both larvae and adult stage. *H. axyridis* showed a high potential cannibalism and aphid predation rate, that did not seem to show a negative impact on the native species.

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Habitat analysis of *Euphorbia* species and associated flea beetles in the *Aphthona* complex from Europe: contributions of ecology studies to biological control

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Habitat associations were characterized for four different spurge species and their associated flea beetle species in the *Aphthona* complex from xeric, mesic, and hygric habitats from 18 field sites in Europe. Micro- and macro-nutrient analyses were conducted on soil, and spurge foliage/roots; physical properties were measured for the soil samples; plant productivity was estimated at each of the sites; and relative abundance counts were made for each of the flea beetle species at each of the 18 sites during spring, early summer, and mid-summer, 1991. Spurge species included *Euphorbia cyparissias* L., *E. lucida* Waldstein-Wartemberg and Kitaibel, *E. seguieriana* Necker, and *E. virgata* Waldstein-Wartemberg and Kitaibel (Euphorbiaceae). Flea beetles species included: *Aphthona cyparissiae* (Koch), *A. czwalinae* Weise, *A. lacertosa* Rosenhauer, *A. nigriscutis* Foudras, *A. pygmaea* Kutschera, *A. venustula* Kutschera, and *A. violacea* (Koch) (Chrysomelidae). The results of ordination analyses and other multivariate approaches revealed that the spurge species and various flea beetle species were each associated with particular chemical and/or physical properties of the soil, chemical properties of the spurge roots/foliage (*Aphthona* spp. only), and levels of plant productivity.

Ordination models, based on the collection of similar data from 48 research sites in the U.S., are being developed in an attempt to validate the the habitat association patterns obtained for the *Aphthona* species from the European data. This information will be helpful in guiding the release of flea beetle species in the appropriate types of habitats in the future, and hopefully will improve their chances for establishment and impact on leafy spurge in North America.

Using decision analysis to assess risk of marine introductions associated with transport vectors

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Marine introductions occur through intentional and unintentional release of organisms from transport pathways such as ballast water, fouling, aquaculture, seafood industry and recreational activities. Managers rely on understanding the relative importance of these vectors as one component in the management of introductions. The discipline of marine invasions is relatively new and documentation is scant on the economic and ecological effects of introduction and the effects of biological control in marine systems compared to freshwater and terrestrial systems. The unknowns and uncertainties challenge managers as they develop regulations and adopt policies to manage, prevent and control marine invasions.

Decision analysis is one approach to assessing the relative importance of risk associated with different transport mechanisms, including biological controls that introduce new species. Decision analysis uses both factual and conceptual information to assist managers in assessment of risk. Using available information, this approach is used to evaluate two transport vectors for introduction of marine organisms. The application of decision analysis to risks associated with the effects of biological control is discussed as a management tool.

Non-target use of native plants by introduced biological control agents of weeds; predictable and avoidable risks

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I tested the hypothesis that biological control projects on target weeds which have closely related native plants in area of introduction will produce more non-target use of native plants by introduced enemies, than will projects against target weeds which lack close relatives in the area of introduction. Close relatives are defined here to be congeneric plants (species in the same genus) and species in closely related genera (those belonging to the same tribe). Non-target use is defined here as the ability of the agent to use the plant as a developmental host, but does not equate with impact which is largely unstudied. Evaluated projects were those conducted in the continental U.S., the Carribean, and Hawaii, and projects in which enemies have been established for at least 6 years in areas where the target weed and closely related native plants co-occur. Most, 57.7 % (15/26), of the U.S. mainland and Carribean projects against target weeds with close relatives have resulted in non-target use of native plants, compared to 0% (0/9) of the projects on target weeds lacking close relatives. At least 34 native plants have become hosts of 10 introduced biological control agents. Most of the native plants that are closely related (congeneric) to the target weeds, however, are not known to be hosts of introduced agents. For instance, 23/90 *Cirsium*, 1/46 *Hypericum*, 0/43 *Salvia*, and 3/63 *Senecio* species that are broadly sympatric with the target weed are hosts. About 20% (10/51) of the agents established against weeds with close relatives have adopted non-target hosts, compared to none of the 17 agents established on weeds without close relatives. In Hawaii, 50% (1of 2) of the weed targets with close relatives resulted in non-target use. The project on *Rubus argutus* Link (Rosaceae) resulted in both native Hawaiian *Rubus* species becoming hosts for the three agents established against the weed. None of the projects against the 18 Hawaiian weeds which lack close relatives resulted in non-target use of native plants, despite the establishment of 45 established agents against those weeds. The hypothesis is unequivocally supported for projects conducted on the U.S. mainland, the Carribean, and in Hawaii. All known non-target use of native plants by introduced biological control of weeds agents has occurred on plants very closely related to the target weeds. In total, 32 of 36 non-target native plants belong to the same genera as the target weeds, while the 4 others belong to 2 closely allied genera in the same tribe. Most, 33 of the 36, of the non-target native plants that have become hosts of introduced agents belong to genera of plants that were used as hosts by those agents in their native areas. The 3 exceptions are species of *Kallstroemia* which have become hosts of the two *Microlarinus* weevils introduced to control *Tribulus terrestris* L., a weed in the Zygophyllaceae. *Kallstroemia* is an American genus of herbs in the Zygophyllaceae, which was previously included in the genus *Tribulus*. Pre-release host specificity testing on weevils indicated that *Kallostroemia* spp. Were acceptable hosts. This data set (involving 103 agents established on 45 weeds) demonstrates that the host specificity breadths of the herbivorous insects and fungi employed for biological control of weeds are highly stable and predictable. The risk to native plants by introduced biological control agents is not related to changes in host range but to biological control practice that 1) targeted weeds with close native relatives, and 2) released of agents with unacceptably broad host ranges. Biological control can be practiced more safely by selecting target weeds which have no or few closely related native plants and by employing agents with suitably narrow host ranges.

Impact of indigenous and exotic parasitoids as mortality factors of *Phyllocnistis citrella* in South Florida, USA

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Seasonal mortality of the citrus leaf miner, *Phyllocnistis citrella* Stainton was studied from 1994 through 1997 on *Citrus latifolia* at Homestead, Fl, USA. Eight species of indigenous parasitoids attacked *P. citrella* in experimental and commercial lime orchards (Browning & Peña, 1995, Peña *et al.*, 1996). The Eulophid *Pnigalio minio* (Walker), a primary ectoparasitoid comprised ≈ 80% of parasitoids. Survival of each developmental host stage and the proportion attacked by indigenous and exotic natural enemies was determined. Before the recovery of the exotic parasitoid, *Ageniaspis citricola* Logvnoskaya in 1995, the third instar host had the highest proportion of parasitized individuals (0.14) followed by prepupa (0.11) while the first instar had the lowest proportion parasitized (0.02). After the first recovery of *A. citricola,* the proportion of pupae parasitized increased to 0.56 followed by prepupa (0.14) and the third instar (0.11). Before the recovery of *A. citricola*, the highest proportion of hosts killed by ectoparasitoids (*i.e.*, *Pnigalio minio, Cirrospilus* spp., Eulophidae) was observed in second (0.17) and third instar (0.15). After the introduction of the exotic species the mortality from generalist parasitoids was greater from second instar (0.31) and third instar host (0.21).

Mortality caused by indigenous natural enemies was significantly correlated with increases of *P. citrella* density. Parasitism of *P. citrella* by the exotic parasitoid, *A. citricola*, correlated less well to host density over the season ($r^2 = 0.12$) than did mortality caused by indigenous natural enemies ($r^2 = 0.76$).

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Integrating pheromone-based and biological controls of the Douglas-fir beetle (*Dendroctonus pseudotsugae*)

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The Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, is the most important insect pest of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, throughout North America. Pheromones are useful tools for manipulating beetle behavior to minimize negative impacts on resource management plans. Pheromones not only influence behavior of the target insect, but also affect natural enemies, particularly predatory beetles.

The Douglas-fir beetle anti-aggregation pheromone, 3-methylcyclohex-2-en-1-one (MCH), has been shown to be highly effective in preventing the infestation of live, high-risk trees during outbreaks. Furthermore, MCH has little impact on natural enemies. Data from several studies indicate that MCH applications to protect trees during outbreaks may actually enhance control by clerid predators. Predator abundance relative to the Douglas-fir beetle was higher on MCH-treated plots compared to untreated plots. There were no differences in abundance of hymenopteran parasitoids or clerid predators in bark samples taken from infested trees on MCH-treated and untreated plots. These data suggest that operational MCH treatments will preserve or enhance important natural enemy populations.

Some components of the Douglas-fir beetle aggregation pheromone are more attractive than others to associated clerids. This presents challenges to developing mass-trapping technologies that will allow for the selective removal of Douglas-fir beetles without adversely affecting predator populations. Conversely, this provides the opportunity to develop operational treatments to enhance clerid populations by aggregating them in areas with abundant host insects. Continuing research is addressing both of these possibilities.

Direct and indirect effects of *Trichopoda pennipes*, adult parasitoid of *Nezara viridula*, ten years after its accidental introduction in Italy from the New World (Diptera: Tachinidae; Heteroptera: Pentatomidae)

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During the last 20 years the number of accidental introductions of exotic insect pests in Italy has increased dramatically and resulted in a variety of situations. In some of these only the exotic pest, without any natural enemy, has invaded the area while in other cases the pest was introduced together with a co-evolved effective parasitoid. A third situation, described here, is the fortuitous introduction of an exotic parasitoid of an indigenous pest, *Trichopoda pennipes* F. and *Nezara viridula* L. (Bin & Bruni, 1997). *T. pennipes,* a tachinid nymphal-adult parasitoid of Pentatomidae and other Heteroptera, is native to the New World and was purposely introduced into several countries with the aim of controlling *N. viridula,* a cosmopolitan pest but probably native to the Mediterranean basin.

In 1988 *T. pennipes* was recorded for the first time in Central Italy due to an accidental introduction together with *N. viridula* (Colazza *et al.*, 1996), and its diffusion was assessed in the following years collecting 4th and 5th instar nymphs and adults of *N. viridula* in different areas of Umbria and Lazio regions. On the west sea coast area, parasitization rate was on average 13% in the two years 1991-92, while in the inland area reached only 4% in the same period. In 1998, *i.e.* ten years after its first record, field observations have been repeated with the same methodology to assess the impact of *T. pennipes* in the old and in other potential areas of invasion. Average parasitization percentage has significantly increased reaching 21.3% on the west coast area (No. *N. viridula* collected = 305) and 24.8% in the inland area (No. *N. viridula* collected = 1681). Bug adults collected from north Italy (Liguria region) and from south Italy (Sicily region) didn't have macrotype eggs of *T. pennipes* on their body. To determine possible host switch of *T. pennipes* from *N. viridula* to other bugs, some other pentatomid species were collected in the field such as *Graphosoma lineatum* (L.), *G. semipunctatum* (F.), and *Dolicoris baccarum* (L.). No adults of these bugs have been found with eggs of *T. pennipes.* The indigenous tachinid parasitoid, *Ectophasia crassipennis* L., has been obtained from *N. viridula* in negligible percentage as in the previous surveys.

The direct effect of this fortuitous introduction is that *T. pennipes* is exerting a more effective control on *N. viridula* since the percentage of attacked adults has increased about 3 times in ten years. Indirect effects, such as competitive displacement of other tachinids or host switch to other bugs, have not been observed so far.

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Host range determination of herbivore insects for classical biological control of weeds: ecological approaches to evaluate the risk to non-target plants

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The host range of *Mogulones cruciger* Hbst. (Col., Curculionidae), a root-mining weevil for the biological control of houndstongue (*Cynoglossum officinale* L.) was assessed between 1988 - 1996. Houndstongue is a herbaceous Boraginaceae species of Eurasian origin that has naturalized on rangelands in western North America.

Field release of the weevil species in North America was recommended by the Technical Advisory Group (TAG) in 1997, and releases were subsequently approved and made in Canada. However, complementary studies on the host range are being conducted to address U.S. concerns, specifically on the environmental safety of releasing *M. cruciger* and on the safety of classical biological weed control in general. The studies comprise 1) an assessment of the natural host range of *M. cruciger* in its native range. At five houndstongue field sites in Europe, plants of seven sympatrically occurring Boraginaceae species were randomly collected and analyzed. Despite severe infestation of houndstongue plants at all field sites none of the sympatric Boraginaceae species was accepted as an alternative host by *M. cruciger;* 2) an assessment of the acceptance by *M. cruciger* of native North American Boraginaceae genera that are not present in Eurasia. We used eight plant species in five genera for starvation experiments. One of the native North American plant species supported the development of *M. cruciger* to a limited degree; 3) studies on differences in the host choice behavior expressed by *M. cruciger* under natural field *versus* laboratory conditions. Plants of four native North American Boraginaceae species and houndstongue were placed at six *M. cruciger* release sites in Canada to determine if *M. cruciger* lays eggs and develops on these plant species. Aside from houndstongue, two of the exposed Boraginaceae species showed feeding signs. In two other experiments, plants of 15 native North American Boraginaceae species and houndstongue were arranged in plots in a common garden and *M. cruciger* adults were released. Two of the 15 Boraginaceae species showed signs of infestation by *M. cruciger*. According to the collected data, *M .cruciger* expressed a more restricted host range under field conditions than it did under laboratory conditions. 4) the monitoring of sympatrically occurring Boraginaceae species at *M. cruciger* release sites in Canada. Samples of two native Boraginaceae species that grow sympatrically with houndstongue were randomly collected and analyzed for signs of *M. cruciger* infestation. One of the two species showed no signs of infestation and one out of 91 plants of the second species contained two larvae of *M. cruciger*.

These studies not only provide specifics on the safety of *M. cruciger*, but will aid in the development of a more accurate and predictive method to assess the host range and potential non-target effects of insects used in classical weed biocontrol.

Incorporating biological control into ecologically-based weed management

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The overall goal of integrated weed management must be to maintain or develop ecologically healthy plant communities that are relatively weed resistant, while meeting other land-use objectives such as forage production, wildlife habitat development, or recreational land maintenance. Land managers must be able to predict the outcome of their weed management strategies in order to optimize economic and ecological benefits and minimize their risks. Once a desired plant community has been determined, an ecologically-based weed management system may be developed. Ecologically-based weed management requires that scientists and managers develop strategies that are based on our understanding of the interactive mechanism and processes (including biological control agents) that regulate vegetation change. Ultimately, the goal is to direct weed infested communities on a trajectory toward more desirable plant communities. Two ecologically based models are proposed: 1) a successional rangeland weed model; and 2) a model based on the life-history of key plants within the community. These models allow the integration of the factors controlling vegetation dynamics. Studies designed to determine the functional relationship between the pre-biological control plant community and the post-biological control plant community can be incorporated into the predictive models. These studies can be conducted by establishing transects radiating from dense weed infested areas in the center of each infestation to an area of low or no weed occurrence on the outside of the infestation. All transects radiate from the center of the same infestation at several sites with different environmental characteristics. Permanent plots can be placed along the transect and sampled for various vegetative parameters prior to a biocontrol release. Biocontrol agents are released throughout the infested area. Once the agents are established, post-release vegetation data are collected. Data are analysed by using pre-release plant community parameters to predict post-release plant community composition using step-down regression procedures. Incorporation of these relationships, along with other weed management (grazing, herbicides) and revegetation/restoration strategies, allow a predictive understanding of their interactive influence on plant community dynamics. These models will provide a method to develop ecologically-based decision support systems for effectively incorporating biological control agents into truly integrated management for weed infested rangeland. In addition, these models will provide the basis for optimizing economic and ecological benefit:risk ratios based on predicted responses to imposed management.

Indirect interactions, food web vignettes, and unconventional biological control

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Biological control was born and reared in the simple world of direct pairwise interactions between species. In middle age, the damnable complexity of ecology has intruded upon this lubberland with foodwebs and indirect interactions among species other than the "agent" and the "target." The canvas of food webs is large, and at this point we have substantial understanding of only one corner, modules of small sets of species interacting through once-removed effects: exploitation, competition, apparent competition and keystone predation are the best known of these modules relevant to biological control. Most ecological communities are more diverse than these modules, and the picture is not unlike a vignette; our knowledge shades off gradually into ignorance of potentially important yet unknown interactions.

This symposium is more academic and abstract than the lion's share of biological control conferences, and I will indulge this liberalism with a discussion of the promise and challenges of two unconventional indirect interactions pertinent to biological control. First, is the novel possibility of biological control of weeds by insect-transmitted diseases; the example is the hypothesized harm to invasive cordgrasses that would be caused by phytoplasmas transmitted by stenophagous planthoppers. The second is the mystery of the natural enemies of entomopathogenic nematodes, which would thwart control of target insect herbivore species by these potent subterranean predators. Both of these indirect interactions present excellent opportunities to explore new frontiers in biological control.

Non-target impacts of insects introduced for biological control of weeds: the New Zealand experience

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Direct non-target impacts can be divided into those which are predicted, and therefore likely to be acceptable, and those that are unpredicted, and may be undesirable. Two examples from New Zealand of the former are cinnabar moth (*Tyria jacobaeae*), introduced for biological control of ragwort (*Senecio jacobaea*), feeding on *Cineraria maritima*, and St John's wort beetles (*Chrysolina hyperici*), introduced for control of *Hypericum perforatum*, attacking tutsan (*Hypericum androsaemum*). Cinnabar moth larvae have also been found on several native *Senecio* species. Two unpredicted direct impacts are the accidental introduction of *Dialectica scalariella* from Australia, and the host-transfer of *Bruchidius villosus* from Scotch broom (*Cytisus scoparius*) to tree lucerne, or tagasaste (*Chamaecytisus palmensis*). *Dialectica scalariella* was released in Australia as a biological control agent for Paterson's curse (*Echium plantagineum*) and has been found in New Zealand on wild populations of the introduced *Echium candicans, E. pininana* and *E. vulgare*, on cultivated *Echium* species, and on *Cynoglossum* and *Symphytum*, all in the family Boraginaceae. It has not been recorded from the widely cultivated *Myosotidium hortensia*, native to the Chatham Islands. *Bruchidius villosus* was first recorded from tree lucerne in 1999 and initial indications are that infestation levels are lower than on Scotch broom. Indirect effects of insects and a pathogen introduced for control of hawkweeds (*Hieracium* spp.) on plant communities have been predicted. Increasing bare ground may be a negative consequence of successful control of hawkweeds (Syrett & Smith, in press).

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Acarological case-study: predator-herbivore-plant interactions

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The phytophagous mites are major pests throughout the world. Their control is very difficult due to the development of resistance against various pesticides. The use of natural enemies is thus essential for any mites control programmes in the plant protection practise of Hungary.

Incorporate beneficial practices are: understanding of the biological and ecological characteristics of the predacious mites in different Hungarian areas. The study was directed to the following questions:

- What kinds of Phytoseiidae and Stigmaeidae species live in sprayed fruit gardens and in the undisturbed areas /Botanical Garden of Szarvas, Körös-Maros National Park?
- Which are the species of Phytoseiidae found in fruit gardens free from pesticides or included in programs of more careful spraying?
- Which are the dominant, endemic Phytoseiidae that can potentially be used in biological plant protection in the Hungarian fruit gardens?
- What are the diversity values in the mite populations of Corylaceae species?
- What are the significant relationships of dendrophilic mite families in the natural areas?
- Which are the direct and indirect effects regulating of densities of phytophagous mite populations?

On the basis of the acarological investigations describing the Phytoseiidae fauna of Hungary's fruit gardens the authors arrived at the conclusion that *Amblyseius finlandicus* Oudemans and *Phytoseius* (*Dubinellus*) *echinus* Wainstein et Arutjunjan can potentially be used in biological plant protection as endemic, dominant predatory mites.

Diversity being a primary marking phenomenon can be used for tracing changes in markings more closely. This is why it can also be used for indicating the phenetical picture of "initial degradation" in the acarological sense of the term. It can characterise the structural relations of a population at a given time by a single number which can be calculated by the "Shannon" - diversity formula. The diversity values of dendrophilic mites living on Corylaceae species range between H"= 0,31-1,78 while their homogeneity values vary between: J"=0,36-0,99 under natural circumstances *i.e.* in environments free from pesticides.

Special attention was paid to the biotic interrelationships within the dendrophilic population complexes. Significant relationships can be found between the following mite families:

Eriophyidae - Phytoseiidae	$Y = 1/(0,11 - 0,01\,LNX)$	r: - 0,36	(P< 5%)
Phytoseiidae - Tetranychidae	$Y = 40,74 + 0,36\,X$	r: 0,39	(P< 1%)
Phytoseiidae - Stigmaeidae	$Y = -8,03 + 0,71\,X$	r: 0,53	(P<0,1%)

Natural mite communities are dynamic systems due to the presence and action of limiting factors such as density, competition, predation etc. and of feedback mechanisms the numbers and individual densities of both phytophagous and predatory species fluctuate around a value regarded as "optimal".

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Evaluation of ecological risk by using exotic polyphagous predators for biological control - laboratory assessment of inter- and intra-specific predation between *Orius insidiosus* (Say) and *Orius laevigatus* (Fieber)

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The attention of many researchers is nowadays focused on how to ensure that the introduction and use of biological control agents for pest control - a key component of sustainable agriculture - is done in a way which does not put at risk non-target organism. The aim of this study is to evaluate the existing interaction between two predators of thrips, the exotic *Orius insidiosus* (Say) and the (European) native *O. laevigatus* (Fieber) by laboratory tests.

Two individuals of the two species were observed in an arena (Ø 8 cm) for 2 hours after the adults had been starved for 24 hours, and the aggressive interactions which occurred were recorded and analyzed. Several combinations among adults and nymphs of both *Orius* species were compared to each other to assess the intra- and inter-specific predation (aggressive interaction rate, predation rate, efficacy of aggression). In a further experiment an adult was put into the arena with newly hatched nymphs during the development time until adult emergence and observations of one hour were carried out three times per day. All combinations of the two species were arranged to evaluate cannibalism and predation. The five experiments were carried out at 25±1°C, RH=70±10%, with constant lighting during observation.

The results obtained show a low propensity for cannibalism and/or inter-specific predation in both the native *O. laevigatus* and the exotic *O. insidiosus* and no difference was observed between the two species. Nevertheless, when no food was available, a certain increase in cannibalism/predation was recorded. Furthermore, adults of both *Orius* species show more frequently aggressive interaction *vs.* the youngest nymphs.

In conclusion *O. insidiosus* did not seem to show a negative impact on the native species. This study could be useful to assess a standard procedure for further control on other predator species.

Presence and impact of introduced and native parasitoids on *Phyllocnistis citrella* Stainton in Greece

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The Citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was first detected in Southeastern Greece in July 1995. It is likely that the insect invaded Greece from the Mediterranean region of Turkey. Then it spread very rapidly and, within a few months, was found in almost all citrus growing areas of Greece.

The damage caused by this insect and the problem which was created led the citrus growers to use chemical control applying a number of insecticide sprays. Chemical control, using broad spectrum insecticides, was a threat which could disturb or even disrupt the Integrated Pest Management of insect pests on citrus, like scales, aphids and *Aleurothrixus floccosus* in particular, by reducing the population density of its natural enemy *Cales noacki.*

To minimize the effect of chemical control against *Phyllocnistis citrella* in the citrus environment an attempt was initiated to control this insect with biological control agents. For this a number of parasitoids were introduced from *Cyprus* in 1996. The introduced parasitoids were *Citrostichus phyllocnistoides*, *Quadrastichus* sp., *Semielacher petiolatus, Ageniaspis citricola* and *Cirrospilus quadristriatus.* Of those *Citrostichus phyllocnistoides*, *Quadrastichus* sp. and *Semielacher petiolatus* were mass reared in insectary of the Institute of Subtropical Crops and Olive in Chania and were subsequently released in Crete (Chania) and Peloponnessus (Argolis, Korinthia, Lakonia). The impact of introduced and released as well as of native parasitoids has been studied since 1996 in these areas, taking samples weekly or every second week.

From the released species, both *Citrostichus phyllocnistoides* and *Semielacher petiolatus* have been recovered up to now. Among the native parasitoids found to parasitize *Phyllocnistis citrella* the species *Eochrysocharis formosa, Pnigalio* sp. and *Cirrospilus pictus* were identified up to now. The species *E. formosa* was the most abundant.

Is host-specificity of biocontrol agents likely to evolve once they are released?

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The possibility of unforeseen non-target effects as a result of post-release genetic changes has long been acknowledged. There are examples in the ecological literature of relatively rapid genetic changes in host-specificity, and it has been argued that biological control agents could be even more susceptible because they are exposed to a new environment where selection pressures might be different and founder effects are possible. I examine evidence of evolutionary change in host-specificity of biocontrol agents, and discuss whether the type and degree of host-specificity can predispose an insect to genetic change.

There are examples of the host-specificity of biocontrol agents changing, but they need not be genetic. Although host-specificity has a genetic basis, its expression in the field can be strongly influenced by environmental factors. For example, the field host range of an insect is frequently narrower than the genetic limits of its host range, namely its fundamental host range. Reasons for this include the insect and potential host not co-occurring, the insect never being sufficiently deprived to accept a poorer host, and the insects' behaviour being biased by prior experience on a particular host. It is therefore important to distinguish between environmental and genetic constraints to host-specificity. A genetic cause for changes in host-specificity has not been demonstrated for any biocontrol agent, although relevant studies are few.

Genetic change in host-specificity is however possible, and an improved understanding of host-specificity will help us assess its likelihood. Host-specificity is a continuum from specialists to so-called generalists. It can be described in two dimensions, host-range breadth (the number of host species and their relatedness) and the relative acceptability and/or suitability of these hosts. The corollary is that genetic change in host-specificity can result in change in host-range and/or change in the relative attack rate on hosts. A change in fundamental host-range is unlikely as it requires genetic novelty. However, if the field host-range is a subset of the fundamental host-range, field host range could change if environmental constraints change. Some of these constraints are influenced by genotype, such as some factors affecting insect distribution. However, direct selection of host-specificity is limited to altering the relative acceptability and/or suitability of existing field hosts. There is evidence that this can occur rapidly and dramatically. Finally, host-specificity may also vary depending on aspects of life-history, such as oviposition, larval development, oogenesis or adult feeding. The more of these aspects that are host-specific, the more genetic constraints there will be to host change.

Although short-term genetic change in host-specificity has not yet been demonstrated for biological control agents, we might expect it to be possible where: i) the field host range is a subset of the fundamental host-range; ii) the field host-range includes non-targets; and/or iii) host-specificity is only specific for one or a few aspects of the insect's life-history.

Evaluating environmental effects of *Encarsia* species (Homoptera: Aphelinidae) for whitefly control in Europe

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Biological control of whitefly pests in greenhouses has become a key component of sustainable horticulture in Europe over the past 25 years. Nowadays, billions of exotic beneficials are produced and released seasonally or inundatvely for the biological control of whiteflies in greenhouses. Although no clear direct adverse effects have been found up till now, the potential non-target effects of these releases have been little emphasised. Here we present some of the ecological effects of releases of exotic parasitoids introduced for the biocontrol of greenhouse whiteflies and we indicate the lines and methodologies of research along which we wish to determine benefits and riks of different types of biological control for agriculture and the environment, to develop reliable methods for assessing the potential risk of import and release of exotic biocontrol agents and to design EU-guidelines to ensure that introduced biocontrol agents are environmentally safe.

Effect of the entomopathogenic fungus *Metarhizium anisopliae* on non-target ground and rove beetles (Carabidae and Staphylinidae) in a lucerne field

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Metarhizium anisopliae was released as a conidial suspension in low and high doses (109 and 6 x 1011 conidia/m^2) to the ground in a lucerne field. One site was sprayed in the spring and another in autumn 1997. Insects, primarily beetles from Carabidae and Staphylinidae were collected from treated and untreated plots, and were diagnosed for the presence of insect pathogenic fungi on the two sites in 1997 and again in 1998.

In each plot 300 - 400 beetles were trapped in pitfalls, but few insects were infected with *M. anisopliae*. The prevalence in Carabidae varied between 0 to 5.7% and in Staphylinidae between 0 to 15.8%, the prevalence in larvae being higher than in adults. Within Carabidae *M. anisopliae* was documented in *Agonum dorsale*, *Carabus nemoralis* and *Amara similata*, and within Staphylinidae in *Staphylinus* sp. and *Oxytelus* sp. In bioassays, larvae of Carabidae proved to be highly susceptible to *M. anisopliae*. *Beauveria bassiana* was naturally present in the lucerne field and caused infection of ground beetles between 0 and 3.5%, while it infected between 0 and 8.5% of the rove beetles.

Before the release of the *M. anisopliae* strain in the lucerne field, 26 and 86 *M. anisopliae* isolates were obtained from selective agar medium and insect baits respectively. After the release, *M. anisopliae* was isolated from 68 ground and rove beetles. Selected isolates were characterized using RAPD and UP-PCR (universally primed - PCR) and at least two different DNA-profiles were found. From the insects one DNA-profile was dominant.

It can be concluded that released *M. anisopliae* affected Carabidae and Staphylinidae in the lucerne field, but only to an extent comparable with naturally occurring entomopathogenic fungi.

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Indirect ecological effects in biological control - the practice of theory

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Interest in the indirect ecological effects of biological control has grown with its increasing popularity, and with a broadening of its constituency beyond the community of biological control practitioners and agricultural pest managers. This interest signals a maturation of the discipline and a need to examine the impact of biological control from a much broader perspective. As an exercise in applied ecology, biological control has always been challenged to predict the outcome of complex population processes. Ecological theory has provided some assistance in understanding success and failure, and now can be usefully turned to examining indirect effects. Doing this reveals that non-target effects bear some interesting relations to early "issues" in biological control, including multiple *vs.* single species introductions and new associations. But more important than theory to the understanding of indirect effects will be sound ecologically-based methodologies for predicting and assessing impact of agents on targets and non-targets.

Ecological and genetic interaction between an introduced and indigenous torymid parasitoids in biological control of the chestnut gall wasp, in central Japan

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The chestnut gall wasp *Dryocosmus kuriphilus*, a serious pests of chestnut tree, is thought to have been accidentally introduced from China around 1940. *Torymus sinensis*, a specific parasitoid of this pests, was imported from China, and in spring 1982, 260 females of *T. sinensis* were released to control the *D. kuriphilus*. This biological control program successfully brought a considerable reduction of damage by *D. kuriphilus* around the release sites. Recently, it has been suggested that *T. sinensis* interacts with *T. beneficus*, an indigenous parasitoid in Japan, and that *T. beneficus* tends to drop off.

We analyzed frequency of hybridization between *T. sinensis* and *T. beneficus* using malic enzyme (ME) locus. Nine individuals of 821 *Torymus* wasps tested showed banding pattern of hybrids: this percentage (1.1%) was much lower than predicted values based on morphological character measures. However, any isozyme markers including ME are unable to perfectly distinguish *T. sinensis* from *T. beneficus*, or their hybrids.

There is still confusion concerning identification of *Torymus* species including the introduced *T. sinensis* and native *T. beneficus*. As of 1982, when *T. sinensis* was first released, only one strain was recorded for *T. beneficus* in Japan (Kamijo, 1982). After that, some ecologically different types (strains) were reported both for *T. beneficus* in Japan (Murakami, 1988) and for *T. sinensis* in Korea (Murakami *et al.*, 1995). To entire understanding the interaction between introduced *T. sinensis* and native *Torymus* species in Japan, we need novel DNA markers distinguishing the introduced *T. sinensis* from all local strains of native *T. beneficus*. Firstly, we aim to indicate affinity among each *Torymus* species or strains, from molecular phylogenetic trees for *Torymus* group distributed in Japan, Korea and China, using a part of the mitochondrial cytochrome c oxidase I gene (COI) sequence.

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Risks in biological weed control: the South African experience

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Biological control of weeds is often criticized because of the inherent risk of damage to non-target species. When beneficial "weed" species of economic importance are targeted for biological control an additional source of criticism is that some or all the benefits of the plant targeted for biological control might be forfeited. This results in conflicts of interest between the users of the plant and those that regard it as undesirable. The decision to release a biological control agent eventually hinges on a risk-benefit analysis in consultation with the stakeholder. Assigning a value to the chances of success of a potential biological control organism or project is difficult but nevertheless an essential part of the equation of decision making. Unpredicted side-effects of a biological control agent, such as host range extension or unwanted dispersal to new geographical areas, cast serious doubt on the credibility of risk assessment and should be avoided at all cost. In our experience, host specificity tests under confined conditions normally result in apparent host-ranges that are far broader than the natural host range of a phytophagous insect, and this provides us with a wide safety margin. All the above issues are illustrated and discussed with case studies from the long history of biological weed control in South Africa involving more than 70 insects and mite species released against 43 weeds, several of which are also of economic importance.

Evaluating indirect ecological effects of biological control, Global IOBC International Symposium, Montpellier, France, 17-20 October 1999. ***IOBC wprs Bulletin*****: 22(2), 1999**

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Le **C.I.L.B.A.** ou Complexe International de Lutte Biologique Agropolis est une structure fédérative d'Agropolis, pôle international de recherche et d'enseignement supérieur dont la spécificité est l'agronomie et le développement des régions chaudes, méditerranéennes, tropicales et subtropicales. Neuf établissements d'enseignement supérieur ou de recherche, établis à Montpellier, ont souhaité organiser l'information réciproque et le développement d'actions communes de recherche, de développement et de formation dans le domaine de la lutte biologique, suivant le concept de développement durable.

Ces neuf établissements fondateurs sont les centres de Montpellier du CIRAD, du CNRS, de l'INRA et de l'IRD, l'Ecole Nationale Supérieure Agronomique de Montpellier (AGRO.M), l'Université de Montpellier-II, les laboratoires américains et australiens de lutte biologique implantés également à Montpellier (USDA et CSIRO), ainsi que la Mission de Coopération Phytosanitaire du ministère de l'agriculture (MCP).

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