Session 12: Environmental Risk Assessment of Invertebrate Biological Control Agents

ESTIMATING PARASITISM LEVELS IN *OSTRINIA NUBILALIS* HÜBNER (LEPIDOPTERA: CRAMBIDAE) FIELD POPULATIONS USING MOLECULAR TECHNIQUES

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Accurate detection and identification of parasitoids are critical to the success of IPM programs to detect unusual variations of the density of these natural enemies, which may follow changes in agricultural practices. For such purposes specific molecular markers to detect *Lydella thompsoni* (Herting) and *Pseudoperichaeta nigrolineata* (Walker) (Diptera: Tachinidae) within the european corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) have been developed. Primers amplifying fragments of the mitochondrial cytochrome oxidase I (COI) gene were designed following alignment of comparable sequences for a range of parasitoid and host species. Each of the primer pairs proved to be species-specific to one of those tachinid species, amplifying DNA fragments of 191 and 91 bp in length for *L. thompsoni* and *P. nigrolineata*, respectively. This DNA-based technique allowed to perform a molecular detection of parasitism in natural populations of *O. nubilalis*.

Molecular evaluation of parasitism was compared with the traditional method of rearing ECB populations in controlled conditions before breaking off the diapause. This method is both labour intensive and slow, taking easily up to 8 months, too long to be compatible with

effective modifications of cultural practices that would be required if unusual variations of densities of parasitoids are detected. Fifth-instars diapausing ECB were collected in maize stalks at twelve sites in three geographical areas of France: three in the South, three in the center and six in the North. For each site, diapausing larvae were randomly assigned in two sets. The first set (fifty larvae per population) was used to estimate the mean percentage of parasitism using this molecular method. The second set was used to estimate the mean percentage of parasitism using the traditional rearing method – i.e., by recording the number of tachinid flies emerged from the ECB larvae. This set contained between 76 and 217 diapausing larvae depending on the population. Obtained percentages of parasitism of both tachinid species were higher – approximately three times – using this molecular method, suggesting an underestimation of the traditional rearing protocol. This study confirms molecular methods as very promising for a correct detection and identification of parasitoids in natural field populations.

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RISK-ASSESSMENT IN OMNIVOROUS PREDATORS

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The fact that many predaceous arthropods can occasionally or regularly feed on plants or plant products is of some concern as if it would inevitably lead to crop damage. However, facultative herbivory does not necessarily cause economic loss in crops. Economic loss is the result of complex interactions between the morphological, physiological and behavioural traits of the predator and environmental features including crop plants. In this poster we summarize the complexity of plant feeding by predators when drawing guidelines for risk assessment of introducing arthropod biological control agents into a new environment.

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EVALUATING THE RISKS TO NON-TARGET SPECIES ASSOCIATED WITH INTRODUCING A STAPHYLINID PARASITOID INTO THE CANADIAN PRAIRIES

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The Eurasian parasitoid *Aleochara bipustulata* L. (Coleoptera: Staphylinidae) is a prospective classical biological control agent for the cabbage root maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae) in Canada. The pest feeds on the roots of *Brassica* plants, and is a growing concern to Canadian canola producers. Prior to introduction, the risks of negative effects on population levels of non-target species will be evaluated by studying the parasitoid's fundamental and ecological host range.

Contemporary protocols for studies on host range of a prospective biological control agent recommend beginning with a review of the literature. The literature review revealed the habitats in which the parasitoid has been found and the host range of the prospective agent and two congeneric species. Researchers have found the parasitoid in fifteen different habitats, and complete development reported within puparia of fly species from seven families of Diptera Cyclorrapha. This information was used to compile a list of dipteran species that both overlap with the habitats of the parasitoid and may support complete development. Species of conservation concern and beneficial species were appended as safeguards. This list was then filtered to include only those species likely to be found in sufficient numbers to permit rigorous testing. This filtered list of non-target species and the habitats reported for the parasitoid in the literature were used to develop a programme for evaluating the risk to non-target species.

Since some reports of habitat use may have been based on improper identification of the parasitoid, pitfall traps will be used to determine if it does in fact exploit some of these equivocal habitats. Collections will be made from natural populations of species on the filtered list to assess the parasitoid's ecological host range. Finally, lab-based studies using species from the filtered list will be used to investigate the parasitoid's fundamental host range.

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EVALUATING PARASITOID HOST RANGE USING MOLECULAR TECHNIQUES: A NEW APPROACH TO NON-TARGET RISK ASSESSMENT STUDIES

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Accurate identification of natural enemies is the cornerstone of biological control and methods that can accurately identify potential biological control agents are essential, particularly when morphological variation among species is slight. Conventional rearing and dissection methods for detecting and identifying parasitoids within their hosts can be tedious and time-consuming; however, these techniques are commonly used in host range and risk assessment studies. Molecular methods for detecting and identifying parasitoids within their hosts have the potential to be both rapid and accurate, and may expedite the ecological studies necessary prior to introducing potential biological control agents. In order to determine the utility of molecular diagnostics in risk assessment studies on European parasitoids of *Lygus* Hahn (Hemiptera: Miridae), a single-step multiplex PCR assay was developed for three *Peristenus* Förster (Hymenoptera: Braconidae) species. The availability of a single-step multiplex PCR assay to detect and identify immature stages of *Peristenus* species within mirid nymphs may facilitate host range and non-target risk assessment studies by eliminating mortality issues encountered using rearing techniques, expediting identification, and providing additional information not available by dissection (e.g. species composition) or rearing (e.g. multiparasitism). The specificity and sensitivity of this assay were tested, and the PCR primers were shown to be highly specific for their respective species and capable of detecting single parasitoid eggs within parasitized *Lygus* nymphs. The single-step multiplex PCR assay was applied to DNA extracted from field-collected target and non-target mirid nymphs from different habitats in northern Germany, and parasitism levels and parasitoid species composition based on rearing, dissection and molecular analysis were compared.

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ASSESSMENT OF SPECIES COMPOSITION OF NATIVE *ORIUS* SPECIES IN JAPAN USING MOLECULAR MARKERS

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The anthocorid bugs, *Orius* spp. (Heteroptera: Anthocoridae), are the most promising biocontrol agents of various minute insect pests, such as thrips. Five species in the genus are commonly distributed in Japan: *Orius sauteri* (Poppius), *O. minutus* (L.), *O. strigicollis* (Poppius), *O. nagaii* Yasunaga, and *O. tantillus* (Motschulsky). *Orius strigicollis* are mainly used commercially for the control of thrips in greenhouses in Japan. However, conservational ecologists are apprehensive that composition of species might be disturbed by the augmentative release. In this study, we developed molecular markers to identify species easily, and surveyed the species composition of *Orius* communities in the field.

Development of molecular markers to identify *Orius* species. By multiplex-PCR techniques using five primers simultaneously, we could identify the five *Orius* species easily. The DNA region amplified was Internal Transcribed Spacer 1 (ITS1) of nuclear ribosomal RNA gene.

Species composition of *Orius* communities. Because *O. strigicollis* has been used in greenhouses throughout Japan recently, released individuals may disperse to the surrounding area. By PCR assessment on over 30 communities however, the species could not be found in the areas where they had not been reported (Fig. 1). This suggests that released *O. strigicollis* has not yet been established under natural conditions.

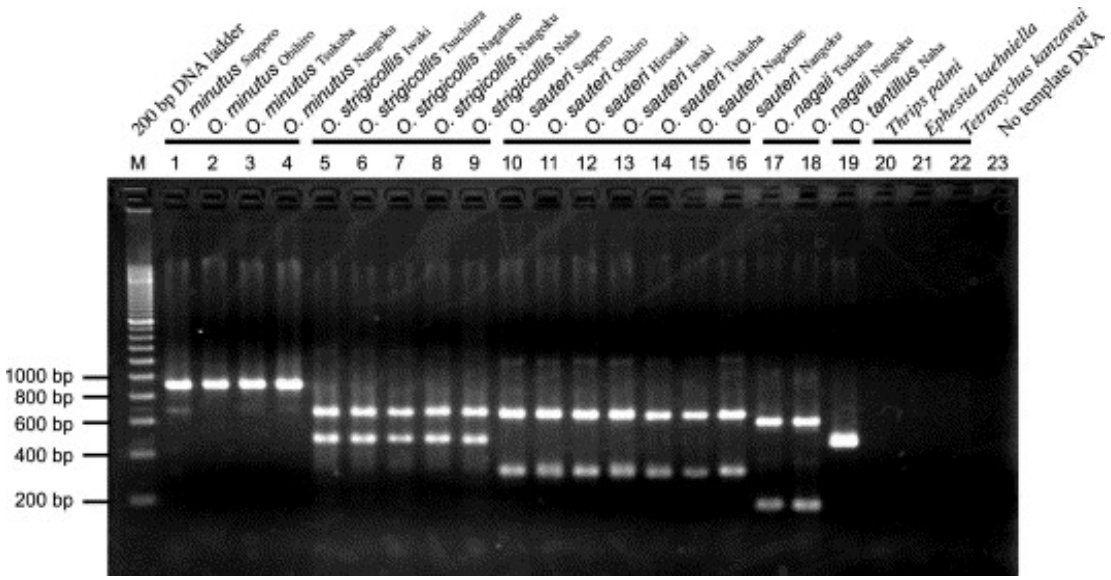

Figure 1. Electrophoretogram of multiplex PCR of five *Orius* species and three prey species. Samples were electrophoresed on 1.6% agarose gels.

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RISK ASSESSMENT OF A POTENTIAL BIOLOGICAL CONTROL AGENT OF CHERRY BARK TORTRIX

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Cherry Bark Tortrix (CBT), *Enarmonia formosana* Scopoli (Lepidoptera: Tortricidae), is a bark-boring pest of several tree species, including *Prunus*, *Malus* and *Pyrus* (Rosales: Rosaceae). Since its accidental introduction from Europe into North America, its high densities pose a threat to nursery and orchard industries as it spreads across British Columbia, Washington State and Oregon State. CBT population levels are consistently low across Europe indicating that this pest has a suite of natural enemies in its area of origin. It was reported that the parasitoid species, *Campoplex dubitator* Horstmann (Hymenoptera: Ichneumonidae), in particular appears to have a substantial impact on European CBT populations and was therefore proposed as a suitable candidate for introduction into North America for the biological control of invasive CBT.

Current regulatory procedures in Canada state that biological control agents must undergo risk assessment prior to their introduction in order to verify minimal risk to non-targets. A new initiative was therefore set up to specifically assess the ecological and physiological host range of *C. dubitator* and to assess potential non-target effects. Experiments were designed according to recently developed guidelines for the risk assessment of biological control agents of arthropod pests and initial tests have been conducted to investigate *C. dubitator* acceptance of selected orchard tortricid species.

It has been indicated in the past that *C. dubitator* shows a strong response to CBT frass and potentially uses it for host location. Preliminary experiments have therefore also been performed with *C. dubitator* to investigate the potential role this indirect cue may play in parasitism of non-target species occupying the same niche as CBT.

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HISTORY AND FUTURE OF INTRODUCTION OF EXOTIC ARTHROPOD BIOLOGICAL CONTROL AGENTS IN SPAIN: A DILEMMA?

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The first documented introduction of an exotic invertebrate biological control agent (IBCA) in Spain occurred in 1908. Since then, 69 additional species have been introduced. We summarize information, both previously recorded and original data, on the species introduced for pest control. Most of the introduced IBCA's focused on citrus pests, and homopterans clearly predominate among target phytophagous species. Success has been more frequent for natural enemies introduced in seasonal inoculative strategies (56.0% of cases) than for those used in classical biological control programs (19.0% of cases). Concerns about potential non-target effects of such species are increasing, but post-release evaluation has often been insufficient to draw conclusions about non-target effects. Most of the beneficial species introduced into Spain were parasitoids (n = 59), and the remaining species (n = 10) were predators. Only 4 of parasitoids are considered specialized monophagous natural enemies. The mean number of host species parasitized by parasitoids is 15.5, whereas the mean number of prey species attacked by predators is 21.2. So, polyphagy seems quite common in the introduced IBCA's in Spain. The rationale guiding many of these introductions in the past would not be acceptable nowadays. Because biological control is such a valuable strategy for pest control, straightforward protocols to evaluate exotic candidate species are urgently needed.

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CAN A PATHOGEN CONSTRAIN THE HOST SPECIFICITY OF A BIOLOGICAL CONTROL ORGANISM?

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Biocontrol is a powerful technology for reducing harm caused by invasive species. It is neither a panacea nor risk-free. Investigators have recently warned that arthropods introduced for biological control of weeds might be harmful to native, nontarget plants, the environment, and public health. Here we ask whether biological control of an arthropod introduced for biological weed control can mitigate the harm it causes to native, nontarget plants. Our case study centers on the decisions to introduce and redistribute the European cinnabar moth *Tyria jacobaea* L. (Lepidoptera: Arctiidae) in North America for biological control of ragwort *Senecio jacobaea* L. (Asterales: Asteraceae). First we show that introduction and redistribution of the cinnabar moth in North America has increased the incidence of herbivory on native, nontarget plants. Second, infection by the microsporidian *Nosema tyriae* reduces the performance (growth, development, survival, pupal mass) of cinnabar moth on test plants in the laboratory. We plan further studies of the incidence and severity of disease in cinnabar moth populations as possible constraints on host use in the field. Finally, we use an epidemiologic approach (spanning 25 sites and 3 years) to show how use of native host plants by this insect is constrained by abiotic conditions, quantity and quality host resources, other specialist and generalist herbivores, as well as natural enemies (*Nosema*). We conclude that biological control introductions are generally irreversible, but it may be possible to mitigate their harmful side-effects.

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ASSESSING PARASITOID HOST RANGES OF CLOSELY RELATED *CEUTORHYNCHINAE* TO AVOID CONFLICTS BETWEEN WEED AND ARTHROPOD BIOLOGICAL CONTROL

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Classical biological control of insect pests and weeds can lead to potential conflicts, particularly if the insect pest and weed agents are closely related. Such a conflict may occur in biological control of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* Marsham (Coleoptera: *Curculionidae*), which belongs to the same subfamily, *Ceutorhynchinae*, as a number of agents introduced or proposed for introduction against invasive alien weed species in North America. The cabbage seedpod weevil is a serious pest of canola and rapeseed (*Brassica napus* L. and *Brassica rapa* L., respectively) in North America. As the future availability of insecticides is uncertain, there is a critical need to more effectively utilize biological control. Several hymenopteran parasitoids of cabbage seedpod weevil are known from Europe. Some of those European parasitoids such as *Trichomalus perfectus* Wlk. (Hymenoptera; *Pteromalidae*) and *Mesopolobus morys* Wlk. (Hymenoptera; *Pteromalidae*) show greatest potential for incorporation into an integrated pest management system for cabbage seedpod weevil in North America. Prior to importation, the host specificity of candidate European parasitoids has to be determined in their native cultivated and non-cultivated habitats to estimate potential non-target risks. The aim of this study is to assess host plant – *Ceutorhynchinae* – parasitoid associations and time of occurrence of *Ceutorhynchinae* and their parasitoids in the fields. This will help to determine potential non-target risks to other *Ceutorhynchinae* implemented as weed biological control agents in North America. A survey in selected crop and non-crop habitats for potential non target insect hosts of *C. obstrictus* parasitoids was conducted at sites in Germany, Switzerland, France, Austria and Hungary between 2002 and 2004. Results of the field surveys as well as parasitoid complexes associated with each selected non-target host will be presented. Results show that *T. perfectus* would probably not attack any native species, or introduced or candidate biological control agent. However, *M. morys* was found parasitizing species such as *Ceutorhynchus turbatus* Schltz., which is a candidate biological control agent of Whitetop (*Lepidium draba* (L.) Desv.). Further studies are being carried out to understand how these results might affect the future decisions in term of introduction of candidate biological control agents into Canada.

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RETROSPECTIVE STUDIES TO ASSESS THE IMPACT OF NATURAL ENEMIES USED FOR CONTROL OF HIBISCUS MEALYBUG, *MACONELLICOCCUS HIRSUTUS* (HOMOPTERA: PSEUDOCOCCIDAE), ON NON-TARGET ORGANISMS

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The hibiscus or pink mealybug (HMB) *Maconellicoccus hirsutus* (Green) (Homoptera: Pseudococcidae) was first reported in Trinidad in August 1995 (McComie 1996). The Ministry of Agriculture, Land and Marine Resources undertook a multi-pronged approach for its management, which involved classical biological control as part of an integrated pest management programme. The programme involved the use of three exotic natural enemies - the Asian parasitic wasp, *Anagyrus kamali* (Moursi) (Hymenoptera: Encyrtidae), the Australian ladybird beetle, *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae) and the Indian ladybird beetle, *Scymnus coccivora (*Aiyar) (Coleoptera: Coccinellidae). Releases of these three biological control agents were made throughout the island of Trinidad following initial releases in February 1996 (McComie 1996).

The hibiscus mealybug has since spread to most islands of the Caribbean and has also been reported in northern Mexico, Belize, Central America and southern California. In most of these countries the HMB has also been kept under control by *A. kamali* and *C. montrouzieri*. A second exotic parasitic wasp *Gyranusoidea indica* Shafee, Alam and Agarwal (Hymenoptera: Encyrtidae) was released in some islands in the Caribbean region to control the HMB. It was introduced in St.Kitts and Nevis, Puerto Rico, Grenada and the United States Virgin Islands through the USDA-APHIS programme. *G. indica* was not officially released in Trinidad, however it was found in HMB infested plant material in Trinidad in the Las Lomas, Arima and St. Augustine areas in early 2000. CABI Bioscience confirmed its identification. At present its distribution is more widespread. The presence of *G. indica* in this country will certainly impact on the HMB Control Programme of the Ministry of Agriculture. Classical biological control is claimed to provide the basis for an environmentally sound solution to pest problems. Thus the general assumption is that there is no risk (non-target effect) associated with biological control since nature is being used to fight nature. Adequate data to defend this has not been systematically gathered. To this end a study has been undertaken in post release monitoring of these natural enemies to determine their efficacy, interactions and impact on non-target species.

The objectives of this study are the determination of the:

1. Impact, if any, of introduced natural enemies on non-target species (Mealybugs) and
2. In the process other Mealybug fauna and their Natural Enemies would be determined.

A general field survey has already been conducted in Trinidad to determine the natural enemies of the local mealybugs. Then this will influence the design of the specific field surveys that are to be conducted to determine the impact of the exotic natural enemies on non-target organisms.

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MOLECULAR DETECTION OF PREDATION BY SOIL MICROARTHROPODS ON NEMATODE BIOPESTICIDES

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Parasitic nematodes have been used as biological control agents on a number of different pests in recent years, including successful control of vine weevils, chafer larvae, leatherjackets, Japanese beetles and slugs. Although effective biological control agents, a number of factors have inhibited the widespread use of nematode biopesticides in agriculture and horticulture. One of these factors has in some cases been the poor persistence of the dauer larvae in the soil soon after application. The persistence of nematode biopesticides in the soil has a large impact on both the short and long term effectiveness of nematodes as biological pest control agents. Although UV light and desiccation are commonly attributed to being the main cause of decline in viable nematode numbers, predation by micro-arthropods in the soil may also play an role. Two of the most common micro-arthropods encountered in soils are mites and collembolans.

Our aim was to develop a set of species-specific DNA primers for the detection of three common parasitic nematodes utilized in biological control, *Phasmarhabditis hermaphrodita* Schneider (Nematoda: Rhabditidae), *Steinernema feltiae* Felipjev (Nematoda: Steinernamatidae) and *Heterorhabditis megidis* (Poinar) (Nematoda: Heterorhabditidae), and test their ability to detect the presence of these nematodes in the guts of micro-arthropod

predators. A 510-bp fragment of mitochondrial CO1 DNA was amplified from each species using general primers and sequenced. From this information, species-specific primers were designed and tested for specificity against a range of non-target organisms.

Feeding trials were conducted using two model organisms representing the micro-arthropod predators, the collembolan *Folsomia candida* (Willem) (Collembola: Isotomidae) and the mesostigmatid mite *Stratiolaelaps miles* (Berlese) (Acari: Laelapidae). After exposure to the nematode prey, DNA from the whole micro-arthropods, including the gut contents, was extracted. Detection of nematode DNA within the gut of both micro-arthropods was possible for a maximum of 12 hours with a half life between 8.1 and 10.2 hours in the guts of *F. candida*. Prey choice trials were then conducted with *F. candida* on soil, in which they were offered three nematode species simultaneously at a range of nematode densities.

The use of PCR and species-specific primers has enabled detection and quantification of predation on scales that were not possible using traditional techniques. Soil ecosystems contain many cryptic food chain links and direct observations of such interactions within the soil are not feasible. The study has proved that predation by soil micro-arthropods on nematode biopesticides can occur in semi-natural conditions and that it is possible they may be a limiting factor on nematode biopesticide persistence in soils.

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ENVIRONMENTAL FACTORS INFLUENCING THE INFILTRATION OF INTRODUCED BIOLOGICAL CONTROL AGENTS INTO INDIGENOUS HABITATS

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During recent years there has been an increase in concern regarding non-target impacts of purposely introduced biocontrol agents on native and desirable species. Considerable effort is being expended to understand the causes of host range expansion. Less effort has been made to address reasons for habitat range expansion. There is a need to elucidate the causes of infiltration of native habitats and the potential disturbance of the community of native arthropods.

Environmental factors influencing parasitoid community and parasitism levels. This study aims to identify key environmental factors that might influence the parasitoid community and parasitism levels by biocontrol agents and adventive parasitoids in remote native habitats in Hawai'i. Two endemic non target insect species, *Udea stellata* (Butler) (Lepidoptera: Crambidae), and *Spheterista infausta* (Walsingham) (Lepidoptera: Tortricidae) are being investigated to quantify the level of parasitism by exotic parasitoids and to explore the effects of environmental variables on the occurrence of the parasitoids. *Pipturus* spp. (Urticales: Urticacea), are the host plants of these insects. These endemic plant species are distributed across a wide range of habitats in Hawai'i, giving the opportunity to investigate various environmental gradients.

CANOCO analysis. We used canonical correspondence analysis (CANOCO) to analyze community structure across gradients (such as elevation, level of disturbance, host plant density, plant species richness, etc). Adventive parasitoids occurred across all environmental gradients, and were most strongly associated with moderately disturbed habitats. Purposely introduced parasitoids were frequently associated with the most pristine and remote habitats. Further analyses are to be conducted to elucidate which environmental factors determine these differences. The results of this study will contribute to the development of risk assessment models for assessing non-target risks posed by potential biocontrol introductions, and will contribute to providing tools to identify habitats that are particularly susceptible to alien parasitoids.

Session 13: Predicting Natural Enemy Host Ranges: Strengths and Limitations of Lab Assays

STRATEGIES FOR EVALUATING NON-TARGET EFFECTS IN ARTHROPOD BIOLOGICAL CONTROL

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Examining possible non-target effects of biological control agents is becoming a more common requirement for many biological control programs targeting arthropod pests. Currently, for classical biological control of weeds, the Wapshere method provides an excellent means for eliminating possible natural enemies that could cause harm to non-target plants. However a rigorous, reliable, and broadly applicable testing standard for arthropod biological control is currently lacking. No-choice and choice testing strategies are a common way to