Proceedings of the

5th International Symposium on Biological Control of Arthropods

Langkawi, Malaysia
September 11-15, 2017

Edited by
Peter G. Mason
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PREFACE

The 5th International Symposium on Biological Control of Arthropods, held in Langkawi – Malaysia, continues the series of international symposia on the biological control of arthropods organized every four years. The first meeting was in Honolulu, Hawaii – USA during January 2002, followed by the Davos - Switzerland meeting during September 2005, the Christchurch – New Zealand meeting during February 2009, and the Pucón – Chile meeting during March 2013. The goal of these symposia is to create a forum where biological control researchers and practitioners can meet and exchange information, to promote discussions of up to date issues affecting biological control, particularly pertaining the use of parasitoids and predators as biological control agents. This includes all approaches to biological control: conservation, augmentation, and importation of natural enemy species for the control of arthropod targets, as well as other transversal issues related to its implementation.

To this end, 14 sessions have been organized in order to address the most relevant and current topics in the field of biological control of arthropods, delivered by invited speakers, contributed talks and poster presentations. Some of these topics have remained as important issues since the first meeting, for example, the understanding of non-target impacts in arthropod biological control and biological control as the cornerstone of successful integrated pest management programmes, underlined by an understanding of the compatability of biological control with pesticide applications. Since the beginning we have also talked about the importance of regulation and risk assessment methodology. This still remains an important topic, but today biological control practioners also need to be better prepared for implementing access and benefit sharing policies relevant for classical biological control practices. But also, as new tools and environmental concerns arise, some fresh interdisciplinary topics have emerged. These days the importance of ensuring that baseline data are in place is far better recognised in order to be able to assess the impact of biological control programmes. This is not only a cost-benefit anaylsis, it also looks at the socio-economic impact of biological control and the effect on livelihoods. In this context, it is also rather important to understand the uptake of existing biological control solutions in low and lower middle income countries in order to be able to formulate strategies to replace the use of highly hazardous pesticides through the use of biological control agents. Ecological questions also remain at the forefront of biological control research. Topics that are currently high on the agenda include understanding the role of native and exotic natural enemies, as well as the importance of pre- and post-genetics in biological control.

Another important goal of these meetings has been to be truly international, and this is why every conference so far has been organized in a different continent. This year we are holding the meeting in Asia for the first time ever and again we have around 150 participants from around the world. As a result, this meeting represents an opportunity for creating and expanding networks between researchers worldwide, in particular researchers from South-East Asia who have not been in the position to attend the preceding meetings.

Thus we expect that the 5th International Symposium on Biological Control of Arthropods will be an important milestone in maintaining forward momentum with arthropod biological control research and practice. In doing so, this will contribute towards improving the sustainability of managed systems and protecting biodiversity on the planet, thus contributing towards the Sustainable Development Goals (Transforming our world: the 2030 Agenda for Sustainable Development).

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Session 1: Accidental Introductions of Biocontrol Agents: Positive and Negative Aspects

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1.1 Accidental Introductions of Natural Enemies: Causes and Implications

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Accidental introductions of natural enemies, including parasitoid and predatory groups, may exceed species introduced intentionally. Several factors favor this: a general surge in international trade; lack of surveillance for species that are not associated with live plants or animals; inability to intercept tiny organisms such as parasitoids; huge invasive host populations in source and/or receiving areas that allow rapid establishment; and lack of aggressive screening for pests already established. Recent frequent and surprisingly rapid accidental natural enemy introductions call into question the regulatory emphasis on a rigorous and protracted process for classical biological control (CBC) introductions, when adventives have a high probability to displace or disrupt this planned process. We provide an overview with three brief case studies.

The volume of global international trade is staggering, and it continues to increase. International shipping moves 127 million containers (TEUs, each ~40m^3 in volume and weighing ~14 tonnes) per year between countries, the majority between continents, for a total of ~5 billion m^3 of freight (2014 totals; World Shipping Council, 2017). About 4x this amount moves domestically in coastal shipping. Additionally, 3.2 billion passenger trips take place by air, and air freight amounts to ~185 million tonnes (about 1/50th of the weight shipped by boat, but delivered in <1 day) (2014 totals; World Bank, 2017). A single adult parasitoid weighs about 1 mg (Harvey *et al.*, 2006), or approximately 70 parts per trillion of a single shipping container – less than a needle in a haystack – and 350,000 such haystacks arrive from foreign ports worldwide *per day*!

Given this massive exchange of merchandise, invading natural enemies are of low to vanishing priority for national authorities inspecting imports for harmful organisms and other threats. Primary concerns are plant and animal pests and pathogens that will do the most serious and immediate damage, not to mention a host of other non-biological concerns such as terrorism, hazardous substances, and material that is illegal, smuggled, and/or counterfeit. In the US Department of Agriculture, the very name APHIS PPQ (Animal and Plant Health Inspection Service, Plant Protection and Quarantine) reflects these priorities, and, aside from known plant and animal pests and pathogens, and their associated carriers, very little else attracts the attention of border patrol inspectors.

Reece Sailer, in a prescient perspective, estimated the number of "beneficial immigrant species" to the US, determining that nearly half (134 of 287=47%) had been accidentally introduced (numbers from his Figure 6, not his text). "As an entomologist specialized in

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introduction of beneficial insects, I find it disconcerting...” (Sailer, 1978). He cited as “valuable” many of the accidentally-introduced species such as San Jose scale parasitoid, *Prospaltella perniciosi* Tower (Hymenoptera: Aphelinidae), and the alfalfa leafcutter bee, *Megachile rotundata* (F.) (Hymenoptera: Megachilidae). A few years later, Sailer (1983) provided a breakdown of the 232 alien “beneficial Hymenoptera,” of which 82 (35%) had arrived accidentally; of the remaining 150, 10 had entered the US from Canada after being introduced intentionally there, and the remainder were intentionally introduced to the US by USDA and University of California scientists.

Roy *et al.* (2011) provide a very thorough recent analysis for alien arthropod predators and parasitoids, based on the DAISIE database for European alien species. Of the estimated 1590 species of arthropods introduced to Europe, 513 (32%) are predatory or parasitic. Of these, 66% were introduced unintentionally. This survey includes a number of groups that would never be considered for CBC introductions, e.g., ticks, fleas, spiders, and social Hymenoptera. Of the parasitoid Hymenoptera, 60 (28%) of the 212 recorded alien species were accidental (unintentional) introductions (Roy *et al.*, 2011-Table 1).

From these two assessements, widely separated in space and time, at least one-third of alien natural enemy species appear to have been introduced accidentally. This is probably an underestimate, given the paucity of knowledge of these faunal groups. Furthermore, the proportion of accidentally introduced species has increased recently, as the number of intentional introductions has decreased, due to more stringent criteria for CBC introductions (Roy *et al.*, 2011-Fig. 3; Hajek *et al.*, 2016a).

Several major invasive pests have been associated with accidental introductions of their natural enemies, with varying outcomes, some still unclear. Below is a brief overview of three examples: gypsy moth, brown marmorated stink bug, and kudzu bug.

Since its discovery in northern Georgia (USA) in 2009, kudzu bug, *Megacopta cribraria* (F.) (Hemiptera: Plataspidae), has been considered a very serious threat to the US soybean, *Glycine max* (L.) Merrill (Fabaceae) crop. Overwintering on kudzu, *Pueraria montana* var. *lobata* (Willdenow) Maesen & S.M. Almeida ex Sanjappa & Predeep (Fabaceae), an invasive woody vine native to Asia, it colonized soy crops and reached very high densities (Gardner *et al.*, 2013) which were very damaging to yields, unless pesticides were applied. In 2013, the scelionid *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Scelionidae) was detected in northern Georgia, and the next year, in 4 additional states (Gardner and Olson, 2016). The origin is unknown and is presumed accidental (Gardner *et al.*, 2013). A CBC assessment for *P. saccharalis* was underway in quarantine at the time of appearance of this adventive population, which was shown to be distinct from the quarantine rearings (W. Jones, personal communication). Meanwhile, as early as 2010 (Ruberson *et al.*, 2013), the cosmopolitan generalist fungal entomopathogen, *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Clavicipitaceae) was noted as attacking kudzu bug, and in 2015, many locations had outbreaks of this pathogen. The pathogen, possibly complemented by *P. saccharalis*, is thought to have caused greatly reduced regional kudzu bug populations (Gardner and Olson, 2016; Blount *et al.*, 2017). It remains to be seen if kudzu bug is vanquished or will rise again in North America.

A second example of a scelionid egg parasitoid accidental introduction is covered in detail by Hoelmer *et al.* (this volume, 1.3). Nearly twenty years after the introduction and spread of the brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) in North America, the Asian scelionid *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) oviposited in three sentinel BMSB egg masses in Maryland, USA (Talamas *et al.,* 2015), and has since been detected in several other eastern and western USA. Bon *et al.* (this volume, 2.6) document at least 3 separate lineages, corresponding to separate accidental introductions of *T. japonicus* into North America. None of these match with cultures held in quarantine for study under a CBC program. Native parasitism has been sporadic and mostly low (Hoelmer *et al*, this volume). However, population declines have been noted in BMSB rearings and in the field, and some of these may be due to a newly-discovered microsporidian, native to North America and pre-dating the introduction of BMSB (Hajek *et al.*, in review). Once again, the plot thickens!

Classical biological control using pathogens (including nematodes) has been infrequently practiced, relative to arthropod CBC introductions. Worldwide, only 70 pathogen species have been introduced for CBC, with a correspondingly low number of 7 species accidentally introduced (Hajek *et al.*, 2016b). However, two of these accidental introductions have played a large role in biological control of invasive gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae) populations in northeastern USA. The first was the introduction of Lymantria dispar multiple nucleopolyhedrovirus (LdMNPV), thought to have been introduced in the early 20th century with parasitoids or plant material, as part of an extended and extensive arthropod CBC effort. During most of the 20th century, this virus, which was later mass-produced and formulated for application by the USDA Forest Service and APHIS, caused epizootics in high-density defoliating gypsy moth populations, resulting in rapid population crashes, and spreading naturally with the host population (Hajek and Tobin, 2011).

The source of the second introduction was initially surrounded by some uncertainty (Hajek *et al.*, 1995). The source of this pathogen was addressed using molecular techniques as well as historical data (Nielsen *et al.*, 2005; Weseloh, 1998) that showed with near certainty that this was an accidental introduction. In 1989, *Entomophaga maimaiga* Humber, Shimazu & Soper (Entomophthorales: Entomophthoraceae) was found in 7 states of the northeastern US. Within 5 years, this fungus had spread to all contiguous states infested by gypsy moth, and host populations in many areas have remained low for most years since. Although released intentionally in 1910-1911, there was no evidence that it established then, and there were many favorable chances to observe the effects of the pathogen in the US between 1911 and 1989 (Hajek *et al.*, 1995; Weseloh, 1998). Another effort resulted in releases in 1985 and 1986, but these were shown to be a different strain and were geographically distant from the 1989 epizootics when *E. maimaiga* was first found in the US (Nielsen *et al.*, 2005).

With the increased focus on guarding against nontarget effects of CBC comes the cost of delay and reduction in number of projects carried out (Hajek *et al.*, 2016a). While this may in some cases prevent negative ecological consequences, criticisms of long-past classical biological control mistakes are today largely misplaced. Calls for more regulation and involvement of all stakeholders (e.g., Blossy, 2016) set up the perfect as the enemy of the good. Practical CBC should strike a balance to solve problems as much as it should seek to avoid creating new problems. With increased delay, perhaps CBC agents and plans may be optimized over more time, and native natural enemies may adapt or intersect with the targeted invasive pest in the interim. More certain though, is the prospect of prolonged and even irreversible ecological and economic disruption from pest damage, pesticide applications, and lost ecological services. Along with delay comes the prospect that accidental introductions of potentially suboptimal natural enemies occur, removing the chance to address pest invasions in a timely manner through best scientific practices.

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1.2 Risks and Benefits of Accidental Introductions of Biological Control Agents in Canada

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Introduction of natural enemies associated with invasive alien species is probably more common than conventional wisdom suggests. Such introductions are usually detected well after the host has established in new regions, sometimes even during or after host range studies have been initiated. In Canada, during the last 30 years, at least seven accidental introductions of natural enemies have occurred in arthropod pest systems (Table 1.2.1). Some introductions have resulted in unforeseen benefits to management of invasive alien species, but also pose potential risks to native biodiversity. Here we focus on two examples of accidental natural enemy introductions of arthropod pests that have had positive effects and potential risks.

Table 1.2.1. Accidental introductions of natural enemies associated with arthropod pests reported in agricultural systems in Canada during the last 30 years.

Agent	Probable year of introduction	Host
Euxestonotus error (Fitch)	1800's	*Sitodiplosis mosellana*
Macroglenes penetrans (Kirby)	1800's [1954 MB; 1984 SK]	*Sitodiplosis mosellana*
Synopeas myles (Walker)	2000's [2015 SK; 2016 ON]	*Contarinia nasturtii*
Platygaster demades (Walker)	2016 BC	*Dasineura mali*
Trichomalus perfectus (Walker)	2009 ON, QC	*Ceutorhynchus obstrictus*
Mesopolobus gemellus Baur & Muller	2007 ON, QC	*Ceutorhynchus obstrictus*
Necremnus tidius (Walker)	1998 BC; 2003 AB, SK; 2007 ON, QC	*Ceutorhynchus obstrictus*

Macroglenes penetrans (Kirby) (Hymenoptera: Pteromalidae) is a key parasitoid that reduces populations of orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), in western Canada. First reported in the 1950's after a wheat midge outbreak in Manitoba and later in the 1980's after a major outbreak in Saskatchewan, conservation of *M. penetrans* has had economic and environmental benefits by reducing

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pesticide use. Although formal host range studies have not been conducted, *M. penetrans* appears to be specific to wheat midge (Doane *et al.*, 2013).

Management of wheat midge incorporates *M. penetrans* parasitism levels (25-46% in Saskatchewan, Doane *et al.*, 2013) into models (Fig. 1.2.1) that provide growers with forecasts of potential crop damage during the growing season. Thus, the accidental introduction of *M. penetrans* has provided benefits through reduced input costs, fewer pesticides being applied, and adoption of practices that conserve natural enemies.

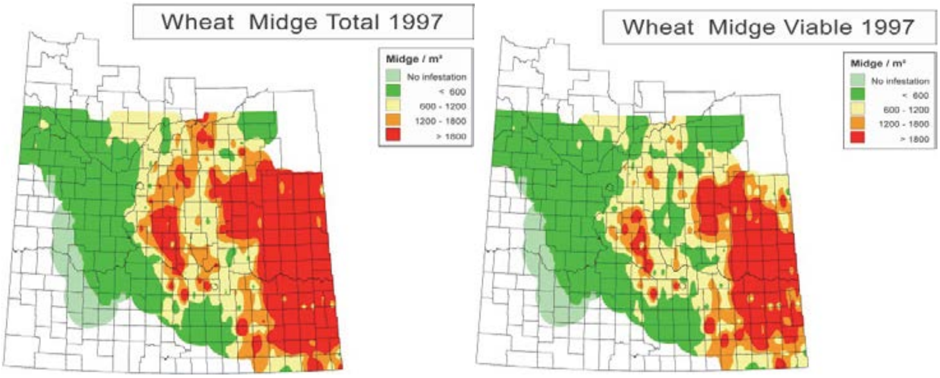

Fig. 1.2.1. Forecast models of wheat midge area infested before (left) and after (right) data where viable cocoons were reduced by *Macroglenes penetrans* to below economic threshold levels (<600/m^2).

Trichomalus perfectus (Walker) (Hymenoptera: Pteromalidae) is an important parasitoid of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), in its native European range and was the focus of more than 15 years of intensive research to assess its potential as a biological control agent. *Trichomalus perfectus* attacks the larval stages of hosts that are concealed within developing siliques of Brassicaceae plants. Non-target species for testing potential impacts of candidate biological control agents were chosen using a multi-criteria selection method (Haye *et al.*, 2015). Parasitism levels (host acceptance by parasitoids) varied among *Ceutorhynchus* spp. and feeding niche (Table 1.2.2). Of the 17 non-target species tested in no-choice laboratory experiments, parasitism by *T. perfectus* of four species was similar to that of the target host *C. obstrictus*. Parasitism of a further five species was lower than that of *C. obstrictus*, and six other species were not attacked at all. Ecological host range surveys in Europe corroborated the prediction that *T. perfectus* would attack *C. cardariae* at similar levels to *C. obstrictus*.

In North America, *T. perfectus* was first discovered in 2009, attacking *C. obstrictus* in Quebec and Ontario and more recently, in a field population of the native weevil *C. omissus*, confirming the prediction that this species is a suitable host. Therefore, based on host range studies, accidental introduction of *T. perfectus* poses a medium to high risk to native *Ceutorhychus* spp., particularly those feeding in the siliques of *Brassica* plants.

Table 1.2.2. Risk of attack by *Trichomalus perfectus* females to non-target weevil species in Europe and North America based on percentage of non-target and target larvae accepted (parasitized) in small arena no-choice tests (host acceptance was compared using Fisher's Exact Test, P< 0.05 (see Haye *et al.*, 2015): high = ns; medium = P<0.05 – P<0.0001; low = P<0.0001; nil = not attacked).

Non-target hosts	**Feeding niche**	**Risk of attack**
Europe		
Ceutorhynchus typhae (Herbst)	seeds in silique	medium
Ceutorhynchus constrictus (Marsham)	seeds in silique	medium
Ceutorhynchus turbatus Schultze	seeds in silique	high
Ceutorhynchus peyerimhoffi Hustache	seeds in silique	nil
Mogulones borraginis (Fabricius)	seeds in nutlet	nil
Ceutorhynchus pallidactylus (Marsham)	stem tissue	low
Ceutorhynchus alliariae H. Brisout	stem tissue	nil
Ceutorhynchus roberti Gyllenhal	stem tissue	nil
Ceutorhynchus erysimi (Fabricius)	stem leaf vein tissue	nil
Ceutorhynchus cardariae Korotyaev	stem & leaf vein gall tissue	high
North America		
Ceutorhynchus americanus Buchanan (on *Rorippa palustris*)	stem tissue	low
Ceutorhynchus americanus Buchanan (on *Sinapis arvensis*)	stem tissue	medium
Ceutorhynchus neglectus Blatchley	seeds, foliage	low
Ceutorhynchus omissus Fall	seeds in silique	high
Ceutorhynchus sp. nr. *nodipennis* Dietz	seeds in silique	medium
Ceutorhynchus querceti (Gyllenhal)	seeds in silique	high
Mononychus vulpeculus (Fabricius)	seeds in pod	nil

Furthermore, *C. constrictus* and *C. cardariae* – which are candidates for introduction as biological control agents of weeds – are also at risk. Thus, although *T. perfectus* may be narrowly oligophagous, monitoring its impact on species at risk will be essential to refine predictions and develop new hypotheses with regards to risks associated with adventive introductions of arthropod biological control species.

Adventive introductions of biological control agents carry both benefits or risks. Understanding the host range of key natural enemies and monitoring them once they are present in the invaded region is essential in managing invasive alien species.

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1.3 Adventive vs. Planned Introductions of *Trissolcus japonicus* Against BMSB: An Emerging Case Study in Real-time

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The invasive brown marmorated stink bug (BMSB), *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), has been responsible for widespread damage to fruit, nut and vegetable crops since its establishment in North America and Europe in the past decade. Further spread to continents that are currently free of BMSB remains a serious risk (Kriticos *et al.*, 2017). Although this insect can also be a pest in its native range in northeastern Asia, its severity appears to be less there than in the newly invaded regions (Lee *et al.*, 2013), and natural enemies of BMSB in Asia are thought to be an important regulating factor. Abram *et al.*, (2017) reviewed surveys for indigenous natural enemies that attack BMSB in the invaded regions, which show that parasitism and predation rates are typically too low to suppress BMSB. Several studies have suggested that these indigenous parasitoids are often physiologically incapable of overcoming host BMSB defenses (Abram *et al.*, 2014, Haye *et al.*, 2015). Successful egg parasitism in particular is much lower than in the native Asian range, suggesting that a classical biocontrol approach to manage this pest may be appropriate. The egg parasitoid *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) (also in literature as *T. halyomorphae* Yang; Yang *et al.*, 2009; Talamas *et al.*, 2013, 2015b) is a key natural enemy of BMSB in its native Asian range (Yang *et al.*, 2009; Qui *et al.*, 2010; Zhang *et al.*, 2017). It has been under evaluation as a candidate biocontrol agent for introduction against BMSB into North America and elsewhere.

Trissolcus japonicus has been reared from several other pentatomid hosts in Asia besides BMSB (Zhang *et al.*, 2015; Matsuo *et al.*, 2016; Kim *et al.*, 2017; Zhang *et al.*, 2017). Laboratory host range testing conducted with no-choice tests in China showed that *T. japonicus* attacked and developed in most of the non-target Asian stink bug hosts tested (Zhang *et al.*, 2017). Similar tests in the U.S. have shown that it will also attack a number of native American hosts, although there is a wide range of developmental success. Choice tests reveal preferences for BMSB in many, but not all, paired comparisons (Hedstrom *et al.*, 2017, KAH unpublished data). Behavioral cues result in additional host selectivity during the process of searching for hosts (Hedstrom *et al.*, 2017).

Recently, several adventive populations of *T. japonicus* were discovered in North America, on the U.S. east coast in 2014 (Talamas *et al.*, 2015a; Herlihy *et al.*, 2016), on the west coast in 2015 (Hedstrom *et al.*, 2017; Milnes *et al.*, 2016), and in 2016, a second population in the northeastern U.S. (Fig. 1.3.1). All three populations are genetically distinct (M.C. Bon, unpublished data). It is not known how they arrived in North America

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but they have established and are expanding their range. Their establishment will allow researchers the valuable opportunity to simultaneously: (1) assess the capacity of *T. japonicus* to impact BMSB populations in an invaded range, (2) determine the host and habitat preferences and fidelity of *T. japonicus* under natural conditions and contrast field results with laboratory evaluations, and (3) study how this introduced parasitoid will interact with resident parasitoids and influence trophic webs.

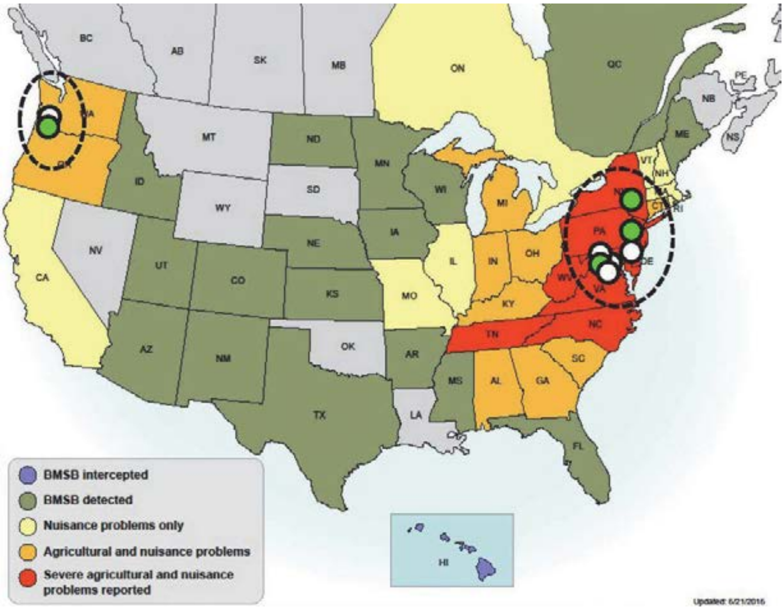

Fig. 1.3.1. Documented field occurrence of adventive *Trissolcus japonicus* in North America (as of December 2016).

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1.4 Can Native Parasitoids Benefit From Accidental Introductions of Exotic Biological Control Agents?

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Interspecific interactions between native and exotic parasitoids can impact community structure and are relevant not only from an ecological standpoint, but also from a biological control standpoint. A thorough understanding of these interactions is critical to estimate the range of potential direct and indirect effects, positive or negative, associated with the establishment of an exotic parasitoid, irrespective of whether its introduction was intentional or accidental.

Exotic pests can be exploited by native natural enemies (food source diversification and/or novel host); however, this exploitation is only adaptive for the native species if it results in enhanced survival and/or reproduction. In environments that have undergone rapid change, previously reliable cues for survival and reproductive success may no longer be associated with adaptive outcomes, resulting in an evolutionary trap that reduces the fitness and reproductive success of the native organism (Schlaepfer *et al.*, 2002). If, for example, a native parasitoid accepts an invasive species as a host but fails to complete development, then the host becomes an evolutionary trap for the native species (Abram *et al.*, 2014). This evolutionary trap could benefit native host species by reducing overall parasitoid numbers, and thus, parasitoid load in those host populations. However, phenotypic plasticity can permit escape from an evolutionary trap if the organism in question learns avoidance behaviour, undergoes morphological changes, or overcomes defensive barriers to development in the host (Berthon, 2015).

The potential occurrence of an evolutionary trap has been associated with the widespread establishment of the invasive pest, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), in Europe and North America. In invaded areas, *H. halys* eggs are readily attacked by native Scelionidae, but are unsuitable for parasitoid offspring development (Abram *et al.*, 2014; Haye *et al.*, 2015). To further increase the complexity of the current system, the exotic Asian parasitoid, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) is being considered for introduction as a classical biological control agent for *H. halys* in recently invaded countries, and has already been documented as an adventive introduction in the USA (Talamas *et al.*, 2015).

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We determined the outcomes of larval competitive interactions between the exotic *T. japonicus* and the European *Trissolcus cultratus* (Mayr) (Fig. 1.4.1), simulating what may happen under natural conditions if both species occupy the same ecological niche (Konopka *et al.*, 2017). Sequential exposure of *H. halys* egg masses to *T. japonicus* and *T. cultratus* at different time intervals demonstrated that native parasitoids can act as facultative hyperparasitoids of the exotic parasitoid, but only during a limited window of opportunity. As such, the secondary invader, *T. japonicus*, could facilitate the use of the primary invader, *H. halys*, as host by a native species, *T. cultratus* in this case, that it would otherwise be unable to effectively exploit as a resource, thus providing a potential mechanism for the native species to escape from an evolutionary trap. In contrast to previously described negative synergistic effects of multiple exotic species on invaded ecosystems, our work suggests that an exotic species could act as an 'invasional lifeline' for resident species, mitigating the negative ecological effects of other biological invasions.

Fig. 1.4.1. *Trissolcus cultratus* foraging on eggs of *Halyomorpha halys* previously parasitized by *Trissolcus japonicus*.

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1.5 Accidental Introduction into Italy and Establishment of *Aprostocetus fukutai* (Hymenoptera: Eulophidae) in Citrus Longhorned Beetle Infestations

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Citrus longhorned beetle (CLB), *Anoplophora chinensis* (Förster) (Coleoptera: Cerambycidae), was accidentally introduced from Asia into 11 countries of Europe and neighbouring states, including Italy, France, the Netherlands, Switzerland, England, Croatia, Germany, Guernsey, Lithuania, Denmark, and Turkey, putting at risk a wide range of broadleaf trees. The destruction of the entire infested trees containing the damaging larval stages of CLB is the preferred method for eradicating the pest. Eradication efforts are mandatory and have been successful in the localities where early detection and rapid action were possible. As of 2017, eradication of CLB has not been achieved in Croatia and Italy. Since 2004, the Plant Protection Service of Lombardy, Italy removed thousands of infested trees. However, in 2015 small residual pest populations were found again in the Parabiago area and the eradication efforts are continuing.

The gregarious egg parasitoid *Aprostocetus fukutai* Miwa & Sonan (Hymenoptera: Eulophidae), which is native to Asia was discovered in CLB infestations near Parabiago, Italy in 2002. Initially, the egg parasitoid was thought to be a new Asian species of Aprostocetus, which was described as Aprostocetus anoplophorae (Delvare *et al.,* 2004). Recent collections of the CLB egg parasitoids in China and Japan, new morphological studies and comparisons with the individuals from Italy, and biomolecular data showed evidence of the synonymy between *A. anoplophorae* Delvare and *A. fukutai*, and Japan was found to be the country of origin of the population established in Italy (Bon *et al.*, unpublished data).

Geographical distribution of *A. fukutai*: In Italy, the parasitoid is not present in all CLB infestations of Lombardy. Its geographical distribution was determined by sampling CLB eggs in the field populations, and by exposing in the field potted sentinel trees containing CLB eggs that had been laid in the laboratory, to attract the parasitoid. In 2010, *A. fukutai* was established in the central area of the CLB infestations around Parabiago, and absent from the other infestations of Lombardy.

Development cycle: *A. fukutai* is a gregarious egg parasitoid of CLB that spends winter in diapause as a full-grown larva in the closed host egg shell. Depending on year (2003-2015),

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emergence of parasitoid adults from the host eggs collected in the field during diapause started in June or early July and extended for one or two months, reaching 50% of the cumulative emergence between late June and late July, which is in synchrony with the peak of egg deposition of its host.

In laboratory rearings of *A. fukutai*, among the CLB eggs that were parasitized in early summer of year 'n', the parasitoid larvae entered diapause in 83.2 ± 2.9% (mean ± SE) of the hosts. Diapause termination took place in late spring of year 'n+1', and the adults emerged in June-July. In the remaining 16.8 ± 2.9% of the host eggs parasitized in early summer of year 'n', the parasitoid larvae did not enter diapause, and a summer generation developed in 49.8 ± 0.6 days (mean ± SE), with adults emerging in late August to early September of the same year. In the field, the presence in early fall of an active population of adult parasitoids was revealed during the exposure of potted sentinel trees baited with CLB eggs: for instance in 2014 at Assago, 47.8 % of the exposed eggs were attacked by *A. fukutai*. In the field and in the laboratory, in all host eggs attacked in September, the parasitoid larvae entered diapause until early summer of year 'n+1'. Thus, both cohorts of parasitoid larvae could enter diapause, which started in mid summer and fall, respectively. There was a statistically significant difference ($P < 0.001$) in the mean duration of development from egg to adult (including diapause) between the two cohorts, which developed in 347.2 ± 0.8 days, and 284.1 ± 1.9 days (mean ± SE), respectively. The main effect of this difference was the synchronization of emergence in early summer of the first *A. fukutai* adults from both cohorts.

Gregariousness and sex ratio: *A. fukutai* females lay a cluster of eggs within a host egg and the parasitoid larvae develop gregariously. The mean number of parasitoids per host egg, calculated from the adults emerged in samples of eggs attacked in the field in Italy was 10.34 ± 0.29 (min = 1; max = 34). The immature stages in a given host egg develop synchronously, and the adults emerge altogether on the same day thru a single exit hole. The mean sex-ratio calculated from the adults that emerged from samples of eggs attacked in the field in Italy was 1 : 5.00 ± 0.29 (1♂ : ♀ ± SE).

Impact of *A. fukutai* on CLB in the field in Italy: In early spring 2008 (during dormancy of immature CLB and diapause of the *A. fukutai* larvae), a sample of 60 stumps of CLB-susceptible trees were collected in a woodlot at Canegrate, and stored individually in emergence bags in quarantine. From the adult beetles and parasitoid adults that emerged in summer 2008, we determined that 38 trees had been infested in 2007 with an overall number of 136 CLB eggs, among which 38 eggs were not parasitized and developed to adult stage, and 98 eggs (72%) were parasitized and produced 1,124 adults of *A. fukutai*. This showed that, in an introduction area, a coadapted Asian egg parasitoid may have a significant impact on an *Anoplophora* species. For comparison, in 2014 in the native range, in an abandoned orchard of pomelos at Meizhou, Guangdong, China, the rate of parasitism of CLB eggs by *A. fukutai* was 79.6%.

Following the substantial CLB eradication efforts by the Italian authorities, the pest population has been heavily depressed, and this has also affected the parasitoid populations. In 2015, exposing potted sentinel trees baited with CLB eggs, and collecting small samples of CLB-infested trees in the historical geographical distribution area of *A. fukutai*, we have shown the persistence of a low level population of the parasitoid in 50% of the sites (4 out of 8 sites) where *A. fukutai* had been abundant until 2010. Thus, *A. fukutai* is still present to attack the pest in the host trees which have yet remained undetected, and it can also serve as an indicator of the presence of residual populations of CLB, where and when the parasitoid is captured in CLB egg-baited sentinel trees.

Host specificity of *A. fukutai*: In 2003, in the laboratory, preliminary no-choice tests were made using logs infested with either CLB eggs, or ALB eggs (Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae)). The logs were caged individually with 3 females of *A. fukutai*. Five CLB eggs were parasitized, while ALB eggs were not attacked. During summer 2003, in a CLB infestation at Parabiago where *A. fukutai* was established, choice tests consisted of exposing, during 2 weeks (4 times from mi-July thru early September), CLB- and ALB-infested logs together in the same plot to attract parasitoids. In 21 logs containing 114 CLB eggs, 13 CLB eggs were parasitized by *A. fukutai*, while in the 29 logs containing 113 ALB eggs, none egg was attacked. During summer 2004 in the same site new choice tests were carried out from June 1st thru mid July: in 41 logs containing 229 CLB eggs, 17 CLB eggs were parasitized, and in 10 logs containing 62 ALB eggs, one ALB egg was attacked. This egg contained 2 parasitoid larvae that reached pupal stage. As one pupa died, and the second did not molt as an adult, a molecular genetic analysis was made before its death. The analysis confirmed strict similarity with the CLB egg parasitoid. However, we considered this unique attack of an ALB egg by *A. fukutai* as a fluke. During summers 2005 and 2006, in the same site new choice tests were made: while some CLB eggs were found parasitized, no other ALB egg was attacked. In 2012, in the laboratory, no-choice tests were made to test *A. fukutai* individuals that emerged from CLB eggs collected in a citrus orchard in Yancun, Guangdong, China. Thirty four *Salix* (Salicaceae) logs were used in these tests, and the target hosts were eggs of CLB, ALB, and *Lamia textor* (L.) (Coleoptera: Cerambycidae) (LT). The logs were caged individually with one female *A. fukutai* for 10-14 days. Twelve logs contained an overall 99 CLB eggs, 11 logs contained 127 ALB eggs, and 11 logs contained 42 LT eggs. *A. fukutai* parasitized 32 CLB eggs, but none of the ALB or LT eggs were attacked. We think the conjunction of these results argue for a strict specificity of *A. fukutai* for CLB.

Moderate dispersal ability of *A. fukutai*, and release technique to spread the parasitoid in isolated pest populations: The moderate individual flight and dispersal abilities of *A. fukutai* seem to be governed by a few endogenous and exogenous factors: the host and the parasitoid females show a similar search pattern for oviposition sites, in that they tend to remain in the same site (one tree or a small group of trees) as long as the resource for oviposition sites is sufficient. The successive generations of CLB females lay eggs in the collar zone of trees that have been previously attacked by their parents, often until no more space with adequate quality bark is available to make oviposition slits. As the *A. fukutai* adults emerge from host eggs that are often located a few centimeters from freshly made egg slits, they do not need to fly a long distance to find hosts. Thanks to its gregariousness and to its apparent sedentary nature, the parasitoid has a high potential to quickly increase its population, and control the pest locally. However, as the dispersal ability of CLB widely exceeds that of the egg parasitoid, the geographical distribution of both insect species tends to be a mosaic of isolated plots with and without presence of the parasitoid. In urban environments, the patchy distribution of the parasitoid is aggravated by exogenous factors like the presence of extended blocks of buildings, industrial plants without a host tree, zones of unsuitable host plants, or other obstacles that affect more the movements of *A. fukutai* than those of the CLB females. In such a situation, intentional introductions of the parasitoid to isolated pest populations could make up for the parasitoid lagging behind its host. The strict specificity of the parasitoid to CLB, and perfect fit with the biology of its host make *A. fukutai* a potentially excellent candidate for classical biological control of the pest where it has been introduced. In the parasitoid-host (*A. fukutai*-CLB) system, which involves a strictly specific parasitoid, the accidental introduction of the parasitoid in Italy was an advantage, but it obviously was insufficient to control the pest, in particular because urban environments create many artificial obstacles to the natural spread of the parasitoid.

We started testing a release technique to spread the parasitoid in isolated CLB populations, using potted sentinel trees infested with parasitized CLB eggs. These were set up in the release sites for the entire diapause of the parasitoid, and thus were subjected to the same climatic conditions as the hosts in the hope to obtain a better synchronization of their emergence. In addition, we recommend to let 2-3 CLB larvae develop in the release tree, which is securely covered with a closed cylinder of strong wire cage with fine mesh (3-5 mm) to retain the emerging CLB adults, and let the parasitoid adults fly away and establish in the surrounding local host populations. On the release tree, the emerged CLB adults feed on the new shoots, mate, and deposit eggs in the collar zone. In this way, some of the newly emerged parasitoids can attack the freshly laid CLB eggs on the release tree, ensuring the occurrence of a second generation of *A. fukutai* including the two cohorts, one not diapausing to release new parasitoids in late summer, and the other diapausing to release new parasitoids in early summer of the next year.

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1.6 Inadvertent Reconstruction of Exotic Food Webs: Biological Control Harms and Benefits

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The movement of exotic and invasive species around the planet continues to accelerate (Hulme, 2009), resulting in a global trend toward homogenization of biodiversity (Capinha *et al.*, 2015). In the process, invasive species are frequently re-connected with accidentally introduced natural enemies from their native ranges (e.g., Colazza *et al.*, 1996; Ramani *et al.*, 2002; Heimpel *et al.*, 2010; Talamas *et al.*, 2015; Medal *et al.*, 2015). These natural enemies are often polyphagous and have the potential to suppress the invasive resource population as well as interact with native species through a variety of direct and indirect pathways. Using a combination of mathematical modeling and experimental work in the lab and field, we examined the case of the introduced aphid parasitoid *Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae) which attacks the invasive soybean aphid in North America. We contrast these findings with other natural enemy-invasive species interactions in the context of biological control risk-benefit assessment.

Aphelinus certus **and the soybean aphid in North America:** The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), was first reported in North America in 2000, and it quickly spread throughout much of the USA and Canada (Ragsdale *et al.*, 2011). The soybean aphid has become the most important insect pest in North America cultivated soybeans, whereas in its native range in Asia it is only an occasional pest (Heimpel *et al.*, 2004; Liu *et al.*, 2004). The 'enemy release hypothesis' is a plausible explanation for this difference in pest status, and an importation biological control program has been pursued. Interestingly, a parasitoid species that was investigated in quarantine, but that was not released due to concerns over risk to non-target native aphids, was eventually introduced inadvertently. This parasitoid, *Aphelinus certus*, was first reported in 2005 in the eastern U.S, and it has since spread so that it now co-occurs with the soybean aphid throughout most of the species' North American range. The introduction pathway for *A. certus* is unknown.

We expanded on host range testing efforts initiated by Keith Hopper (at the U.S. Department of Agriculture) (Hopper *et al.*, 2017), and evaluated 18 aphid species as potential hosts for *A. certus* in the laboratory. We confirmed that *A. certus* can develop in several native aphid species, and documented parasitism of some non-target species, by *A.*

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certus in the field. We conclude that the decision not to release *A. certus* was well-founded. However, using a field exclusion cage experiment, we demonstrate that population growth of the soybean aphid is significantly reduced when in the presence of *A. certus* at ambient field densities. *Aphelinus certus* has become the overwhelmingly dominant parasitoid attacking the soybean aphid in areas where we conducted surveys (Minnesota, USA). Moreover, during a three-year field study, we observed that *A. certus* populations tracked very closely to soybean aphids over a wide range of densities ($R^2 = 0.90$ for a simple linear regression of ln-transformed densities). Thus, biological control of soybean aphid by *A. certus* is likely already resulting in less insecticide use by growers that utilize action thresholds.

Modeling biological control risks and benefits: We employed a general mathematical modeling approach to further evaluate conditions that may influence risks to non-target species versus benefits to biological control of pests (Kaser and Heimpel, 2015). Model simulations demonstrate that a range of outcomes are possible and that natural enemy-mediated indirect effects may vary dramatically depending on which factors delimit host range (i.e., preference versus performance). Apparent competition can result in a biological control benefit, even at low levels of non-target impact. However, if a parasitoid experiences egg limitation, high oviposition rates on resistant non-target hosts can dramatically decrease biological control efficacy while still causing significant harm to non-target populations (Kaser and Heimpel, 2015).

Recently, we expanded upon this modeling effort to evaluate possible natural enemy-mediated indirect impacts of non-reproductive mortality (i.e. cases where the host dies but the parasitoid does not successfully reproduce). We pursued this investigation in part because parasitoid-induced host egg abortion – a phenomenon that occurs when both the host egg and the developing parasitoid die – appears to be common for native scelionid egg parasitoids attacking the invasive brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) (Abram *et al.*, 2014, 2016). We found that host egg abortion can have important biological control benefits if the parasitoid population can be maintained on other suitable hosts species in the community. We also note that *H. halys* represents another case where a parasitoid, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) being evaluated for importation biological control was introduced inadvertently (Talamas *et al.*, 2015).

Conclusions: The case study involving the soybean aphid and *A. certus* clearly demonstrates the tradeoffs involved in biological risk-benefit assessment. *A. certus* presents a risk to native North American aphids, but if it contributes to a large reduction in soybean aphid populations, there may be ecological benefits as well. For example, populations of the invasive harlequin lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), are strongly linked to the soybean aphid in North America (Bahlai *et al.*, 2015), and reductions in *H. axyridis* populations would likely benefit native aphids as well as native coccinellids. There have recently been a handful of cases in which an exotic pest has reconnected with a parasitoid coincidentally being studied for importation biological control (Heimpel *et al.*, 2010; Talamas *et al.*, 2015; Medal *et al.*, 2015). These cases present opportunities to increase our understanding of invasion biology and improve our chances of successful importation biological control. Natural enemy-mediated indirect effects are common in nature, and they may be particularly important in understanding the potential impact of polyphagous natural enemies that successfully develop on abundant invasive species.

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Session 2: The Importance of Pre- and Post-Release Genetics in Biological Control

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2.1 Practical Management of the Genetics of Classical Biocontrol Introductions

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In classical biological control projects the goal is to establish a natural enemy from the native range of a pest insect into an area where the pest has arrived without its natural enemies and has reached pest status. The conditions in this invaded range generally will differ from the native range, consequently the introduced natural enemy will have to adapt to these novel conditions. During the process of introducing a natural enemy into an invaded range many different steps are taken that can influence the ability of this natural enemy to adapt to the novel circumstances. In general, the ability of a population to adapt to new circumstances is a function of its genetic variation, the more genetically variable a population is the more likely it is that it will be able to adapt to the novel circumstances. Consequently, maintaining genetic variation is thought to be important for success in classical biological control introductions.

The topic of maintaining genetic variation in classical biological control programmes has been reviewed at regular intervals over the last 40 years (Hopper and Roush, 1993; Hopper *et al.*, 1993; Mackauer, 1972; Nunney, 2003; Roush, 1990a, 1990b), and it is clear what conditions cause the loss of genetic variation. The first reduction in genetic variation takes place when the natural enemies are collected from the field in the native range. Here the guiding principle is that the more unrelated individuals are collected in the native range the higher the genetic variation will be in the founding population.

The founding population often needs to be reared in captivity for a prolonged period to satisfy quarantine and non-target testing requirements. The larger the population the slower the loss of genetic variation. However, it is often difficult to keep captive populations at large population sizes simply because the problems that may occur in providing enough host material to the natural enemy. Failures in this provision will inevitably lead to often prolonged maintenance at low population numbers. Yet, even if populations can be kept large a second problem will also reduce the ability of the population to perform properly in the field and that is domestication (Woodworth *et al.*, 2002). Domestication takes place when genetically variable populations adapt to the captive rearing conditions. The domesticated natural enemies, while doing very well under captive rearing conditions, often do not perform well under field conditions (Woodworth *et al.*, 2002). A final problem associated with captive rearing is relaxed selection, where traits that are important to the field but are not during the captive rearing, will not be selected during the captive rearing and may be lost through drift.

In the reviews of these negative effects of captive rearing many solutions have been suggested to avoid the genetic problems described above. For parasitoid wasps the solutions for many of these genetic problems are clear. Maintaining isofemale lines of many field collected mated females results in a frozen genetic makeup within these lines and no changes are expected over time, except through mutations (Roush and Hopper,

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1995; Stouthamer *et al.*, 1992). Since within each isofemale line variation is lacking no domestication or relaxed selection can take place. A large part of genetic variation present in the native range can be restored by mixing wasps of the lines and recreating a population. This population can then be used for establishment in the field. Maintaining isofemale lines has the advantage that even if the number of individuals is that is kept in each line is small, no additional loss of genetic variation will occur. Thus, at times when host material is scarce in the rearing program, it will not result in additional loss of genetic variation as long as all isofemale lines remain alive.

For many parasitoid hymenoptera this is a method that can be applied because the wasps are capable of withstanding inbreeding without problems. In wasps with the complementary sex determination system (Ichneumonoidea) this may be a problem. Inbreeding in these groups will lead to the production of diploid males from fertilized eggs. Such lines may be much more difficult to maintain, particularly if the diploid males mate and their sperm is used to fertilize eggs (Stouthamer *et al.*, 1992). For such species it may be best to try to maintain several large populations that are isolated from each other. Within each of these large populations the loss of sex alleles will be reduced, but domestication and relaxed selection will take place. However, by mixing several of these these large populations in mass rearing before their offspring is released in the field many of these rearing induced problems may be avoided (Woodworth *et al.*, 2002).

Once the released genetically variable natural enemies have established for a year, they can be recollected to exploit their genetic adaptation to the local circumstances. These recollected lines can then be used as the starting material for the second phase of mass rearing using recollected, locally adapted, lines as the basis for the mass-rearing process.

Discussion: Until now, as far as I know, this approach has not been used in any classical biological control project, with exception of the release of *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) for classical biological control of the Asian Citrus Psyllid in California. Why not? Several reasons will be discussed below.

1) Outside of theoretical considerations there is very little direct evidence from biological control programs because it is very difficult to test in the field. However, in mass rearing for Sterile Insect Technique (SIT) applications domestication of insects during mass rearing is known to negatively influence the effectiveness of SIT. In the mass rearing for Melon fly it was noticed that, after as little as eight generations of mass rearing, the flies had adapted to the mass rearing conditions by faster development, that then let to a change in daily activity pattern and a loss of synchronization of mating time of the mass reared flies and the wild flies (Miyatake, 1998). 2) It is too costly to maintain separate lines. Maintaining separate lines in many cases will not require much work. At best, one worker who will spend part of their time maintaining lines. This extra cost compared to the overall cost of a prolonged biological control project is negligable. 3) Other arguments that have been used are: some of our classical success stories in biocontrol did not pay any attention to genetics and yet were very successful. The contra argument to this is: why are so many classical biocontrol projects unsuccessful? In conclusion, I would like to cite Nunney (2003) who stated: *"Releasing genetically depauperate stocks .... does not guarantee failure, but it can be expected to minimize the chance of success"*

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2.2 Genetic Diversity of Field and Laboratory Populations of *Mastrus ridens* and Consequences of Inbreeding During Laboratory Culture

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Classical and augmentation biological control depend on successful rearing of natural enemies in laboratory. Factors such as founding population numbers and genetic composition, in combination with culturing procedures and time in captivity, can affect the persistence and quality of a laboratory colony (Woodworth *et al.*, 2002; Gilchrist *et al.*, 2012; Francuski *et al.*, 2014), which might also determine its success in achieving pest control once released in the field (Hopper *et al.*, 1993; Taylor *et al.*, 2011; Fowler *et al.*, 2015). Additionally, in classical biological control programs promising natural enemies of key pests are often transferred from laboratory to laboratory. Populations associated with these transfers typically experience bottlenecks, either because numbers transferred are small or because they suffer high mortality rates at the beginning of their establishment under new rearing conditions. Thus many laboratory colonies may experience strong founder effects, loss of genotypic variability and increased inbreeding, but the effects of these processes on the establishment or efficacy of the field-released individuals is not clear, because rarely data on population genetics of natural enemies is collected during importation, quarantine and rearing (Hopper *et al.*, 1993; Hufbauer and Roderick, 2005).

Mastrus ridens Horstmann (Hymenoptera: Ichneumonidae) is a specialist gregarious ectoparasitoid of the codling moth, first imported from Kazakhstan to the USA in the 1990's and then moved from laboratory to laboratory worldwide for the next 20 years (Mills, 2005; Devotto *et al.*, 2010; Sandanayaka *et al.*, 2011a, 2011b; Tortosa *et al.*, 2014; Retamal *et al.*, 2016). Additionally, this is a haplodiploid species with complementary sex determination (CSD) (Retamal *et al.*, 2016), making it an interesting biological model to study the evolution of populations used in classical biological control and the consequences of inbreeding in captivity.

Field collections were carried out near Almaty, Kazakhstan in 2013 and 2015, in the same area where Mills and collaborators collected the parasitoid in the 1990´s (Mills, 2005; Zaviezo *et al.*, 2014). Additionally, individuals from the mass rearing colony of New Zealand (formed by descendants of *M. ridens* collected by Mills), were imported to the

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laboratory in 2014. We developed microsatellite markers (Retamal *et al*., 2016) to compare the neutral genetic diversity of the population collected in the field in 2013 and several laboratory populations. Microsatellites were also used to evaluate the occurrence of diploid males. Later, we performed experiments to study the consequences of inbreeding in laboratory. For that, a stock population was created mixing individuals from our laboratory colonies, and from them we derived our parental generation. Then controlled crossings between sibling and non-sibling individuals were performed for five generations and life history traits and male ploidy were monitored.

We found that genetic diversity was highest in the wild collected individuals and lower in populations that have been longer in captivity and had more transfers. Percentage of diploid males in these populations ranged from 4 to 30%, with the proportion of diploid males in laboratory populations being significantly larger than in the field population (details in Retamal *et al*., 2016). Experiments showed no effects of inbreeding on several female life history traits (i.e. longevity, parasitism, fecundity), but females in the inbred lines produced less daughters, a more male biased sex ratio and a higher proportion of diploid males. Diploid males were fertile, but females mating with them produced less daughters.

The results of this research show that parasitoids maintained in rearing for long time and transferred between laboratories can suffer genetic diversity loss. Results also highlight the importance of determining the type of sex determination in parasitoid wasps used for biological control and that maintaining genetic diversity and avoiding inbreeding is important when rearing parasitoids species with CSD. This research also shows that molecular tools should be implemented more frequently in classical biological control programs.

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2.3 Effects of Genetic Diversity, Inbreeding and Outbreeding Investigated in Six Reared or Released Biocontrol Agents

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Phenotypic traits relevant for efficient mass-production or field use of biological control agents may be affected by the genetic composition of individuals and populations. The genetic load of populations may for example negatively affect fitness when sampling or rearing strategies generate inbreeding and thus increase genome homozygosity (i.e., leading to inbreeding depression). Outbreeding, for instance created by intraspecific hybridization, may also have a range of effects on populations, from fitness increase due to heterosis effects and more generally related to the creation of beneficial new allelic combinations, to fitness decrease due to disrupted local adaptation or pre- or post-zygotic reproductive isolation. In addition, increased population genetic diversity is supposed favourable for biocontrol as it may increase adaptability of populations to new environmental conditions, be it in production settings or in the field. Although several reviews and opinion articles discuss how genetics matter in biological control or could be used for its improvement (Hopper *et al.*, 1993; Hufbauer and Roderick, 2005; Mackauer, 1976; Nunney, 2003; Roderick and Navajas, 2003), experimental data remain scarce (for example, see review in Fauvergue *et al.*, 2014). Practical applications taking profit of genetics in classical biocontrol or in the industry for augmentative biocontrol are also very uncommon.

Here, we report the main results of studies that have investigated the effects of population characteristics (genetic diversity) or genetic processes (inbreeding, outbreeding) on phenotypic traits or population establishment in six biocontrol agents : *Allotropa burrelli* Muesebeck (Hymenoptera: Platygastridae), *Chrysoperla* near *comanche* (Neuroptera: Chrysopidae), *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae), *Psyttalia lounsburyi* Silvestri (Hymenoptera: Braconidae), *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) under laboratory or field conditions.

Sensitivity to inbreeding was studied in *A. burrelli*, *M. pygmaeus*, *C.* nr *comanche* and *C. montrouzieri* by comparing phenotypic traits between individuals coming from sibling crossings (inbred treatment) or non-sibling crossings (outbred treatment). Differences between inbred and outbred individuals were observed on male longevity in *C. montrouzieri* and on reproduction success in *M. pygmaeus* (Fig. 2.3.1), outbred individuals displaying 30% higher reproduction success.

Impacts of outbreeding, via intraspecific hybridization, were investigated in *C.* nr *comanche* and *M. pygmaeus*. Various field populations were sampled and maintained separately in the laboratory for a few generations. Then a crossing experiment was carried out, with crossings (i) within field-sampled populations of origin or (ii) between field-

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sampled populations of origin. In both cases, experiments were complicated by the occurrence of cryptic taxa. In *Chrysoperla* sp., reproductive isolation prevented from reaching sufficient numbers of replicates to generate reliable results. In *M. pygmaeus*, detailed analyses taking profit of genotyping-by-sequencing data revealed the occurrence of three genetic clusters displaying high reproductive isolation between each other. However, analyses investigating the relationship between inter-individual genetic distance and reproduction success or progeny phenotypic traits revealed a non-linear relationship between genetic distance and reproduction success (with distinct ranges of genetic distance preferentially associated to success vs failure of reproduction). Within genetic clusters, female heterozygosity rates were found positively correlated to the number of offspring produced.

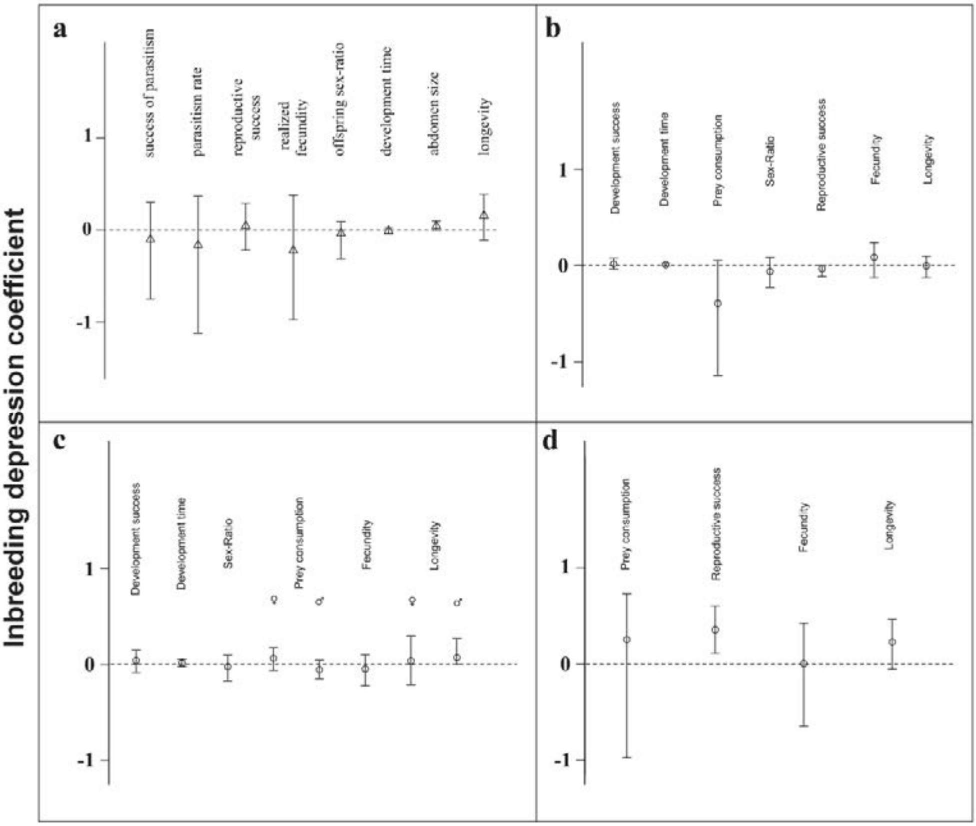

Fig. 2.3.1. Inbreeding depression coefficients (and confidence intervals) for several traits measured in **a)** *Allotropa burrelli*, **b)** *Chrysoperla* near *comanche*, **c)** *Cryptolaemus montrouzieri*, **d)** *Macrolophus pygmaeus*.

Impacts of initial genetic diversity on population establishment in controlled conditions or in the field were studied in *P. lounsburyi*, *A. burrelli* and *T. brassicae*. For *P. lounsburyi*, several population samples were directly imported from South-Africa and Kenya, introduced in laboratory conditions and monitored over several generations. The influence of initial genetic diversity was studied via the correlation between the initial microsatellite allelic richness and the time before extinction of populations. A positive relationship between population initial genetic diversity and time before extinction was observed (a significant impact of the initial *Wolbachia* infection status in the introduced populations was also detected). For *A. burrelli*, populations were created from available lab-maintained material and introduced in laboratory conditions. Extinction rates and population densities over three generations were compared between two treatments: (i) populations created from a unique laboratory line with a fixed genotype vs (ii) populations created from a mix of five lines each with a fixed genotype. No difference was detected between *A. burrelli* populations created with several vs one genotypes. For *T. brassicae*, populations consisting of a unique line with fixed genotype or mixes of lines were released in field conditions and their dispersal was evaluated over a few days. Higher dispersal and parasitism rates were observed when populations consisting of mixed lines were released.

These results lead us to underline the following messages: 1) these results together with other works on arthropod taxonomy reveal that cryptic taxa are common in genera used in biological control; this makes particularly risky most practices consisting in adding field-collected individuals to rearings to improve genetic diversity; 2) although experiments can provide practitioners with valuable data on their specific biological material, producing results of generic value is difficult because results are very dependent on the characteristics of the populations used in each experiment (e.g., variations in the genetic load, homozygosity rates, etc.); moreover, expected sizes of effects are relatively small, making it mandatory to design experiments with numerous replicates and detailed statistical test power analyses; 3) although still demanding in terms of expertise in bioinformatics, genomic analyses are getting more and more accessible for biocontrol and are advantageous because they can, to some extent, decrease the need in terms of number of experimental treatments and replicates to reach conclusions about the impacts of genetics on populations; 4) concrete actions dealing with the management of genetics are getting adopted by companies, and R&D perspectives are defined for some highly used biocontrol agent species.

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2.4 Rapid Biocontrol Evolution in New Zealand's Species-sparse Pasturelands

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This contribution discusses the implications of evidence for the loss of efficacy of the parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) against the exotic weevil pasture pest, the Argentine stem weevil (ASW) *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) in New Zealand. This decline has occurred to the extent that increased weevil damage levels are now being reported (Popay *et al.*, 2011). Of particular significance is the parthenogenetic reproduction of the parasitoid versus the sexual reproduction of ASW. While the weevil can adapt via genetic recombination associated with sexual reproduction, the same does not apply to the clonal parasitoid. This may have led to what is sometimes called an 'unequal evolutionary arms race' (e.g., Goldson *et al.*, 2015). To explore this further, the genotypes of both the parasitoid and the weevil are being investigated using the method known as genotyping-by-sequencing (J. Jacobs**,** unpublished data).

In addition to this apparent reproductive asymmetry, it has been postulated that New Zealand's pastoral ecosystems have also contributed to the acquisition of resistance (Goldson *et al.,* 2014; Tomasetto *et al.,* 2017). In spite of the superficial appearance of similarity, New Zealand's pastoral ecosystems are very different from their Palaearctic-equivalent grazed grasslands. The 'improved' sown pastures in New Zealand, covering about 40% of the land area, originally comprised indigenous forest and native grasses which have been cleared over the last 150 years. The sown pastures now comprises incomplete assemblages of deliberately and accidently introduced species. More specifically, many of New Zealand's pastures are dominated by perennial ryegrass**,** *Lolium perenne* L. (Poaceae) and white clover, *Trifolium repens* L. (Fabaceae). This combination has been and remains, the most cost-effective and sustainable means of providing forage for the country's livestock industries. Thus New Zealand pastures are in effect, a partial and very incomplete transplant from elsewhere and as such, cannot be considered to be 'evolved' ecosystems such as are found in the Palaearctic. Pasture ecosystems, e.g., in the UK, effectively occupy gaps in a predominantly sylvan landscape. Taken as a whole, British landscapes comprise a continuum of invertebrate species across both the grassland

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and forest ecosystems in start contrast to New Zealand's abrupt boundary between its indigenous and exotic habitats between which there is little interchange of species (Brockerhoff *et al.*, 2012).

Exotic pest populations in New Zealand's grasslands often build up to very high and damaging densities, despite these species being of minor significance in their native ranges. For example, ASW can scarcely be detected in its natural range, whereas in New Zealand they can reach densities of up to 700 m^{-2} (Barker and Addison, 1993). Part of the reason for this is likely to be the absence of natural enemy guilds in New Zealand's grasslands (e.g., Goldson *et al.*, 1997); this is an example of the 'enemy release hypothesis' (e.g., Hong and Stiling, 2006). The extraordinary lack of co-evolved diversity in the New Zealand is highlighted by the fact that there are 117 *Listronotus* spp. in temperate South America (Donato *et al.*, 2003) compared to New Zealand's one.

UK (2017) lists the numbers of significant natural enemy species found in UK crop and grasslands allowing a comparison to be made with similar estimates for New Zealand's grasslands. For many taxa the numbers of species of natural enemies are far lower in New Zealand suggesting less natural enemy diversity. For example, in the UK there are 274 species of Syrphidae (Diptera), compared with about 45 species in New Zealand. Tellingly, only ten of these New Zealand species are estimated to occur in pastures. Related to this, Thomas *et al.* (1992) and others, have shown that in the UK, natural enemies tend to overwinter in the boundaries of fields and then migrate into the pasture/croplands in spring, consistent with the idea of British woodland and grassland being an ecological continuum. In New Zealand, however, while there is considerable indigenous natural enemy diversity in the native ecosystems, much of it does not move into the country's agro-ecosystems. McLachlan and Wratten (2003), have shown this clearly with predatory spiders.

The success of classical biological control in New Zealand pastures dealing with invasive weevil species has been extraordinarily high. Barlow and Goldson (1993), Basse *et al.* (2015) and Barker (2013) have shown high levels of control of *Sitona discoideus* Gyllenhal in lucerne *Medicago sativa* L., *S. obsoletus* (Gmelin) (Coleoptera: Curculionidae) in clover and ASW in pasture. This has all been via species and ecotypes of *Microctonus*. Such success in all three biocontrol programmes is unexpected since historically, the chance of a successful biological control initiative has been estimated to be about 10% (Gurr and Wratten, 2000). Thus, in New Zealand the chance of this result was 1:1000. Such success again, can be attributed to the lack of ecosystem complexity and consequently parasitoid natural enemies (e.g., hyperparasites). The very thing that allowed the pest species to thrive also allowed the parasitoid species to thrive, resulting in the recorded very high levels of parasitism.

Thus, there has been strong selection by *M. hyperodae* on the ASW, in the absence of other interfering or competing species. This effect has probably been been heightened by the uniformity of New Zealand pastures, that preclude the provision of either spatial (e.g., Hanski, 1981) or temporal refugia (e.g., Godfray *et al.*, 1994) for the weevil host. In summary, the unequal evolutionary arms race has been accentuated by the pastoral ecosystem itself. New Zealand's farmlands are typical of an island ecology affected by invasive species. It is therefore perhaps significant that the only other recorded example of similar rapid evolution in response to parasitim has been in Hawaii, where a cricket became resistant to a parasitic fly (Pascoal, 2014).

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2.5 Food Webs, Multiple Enemies and Biological Control

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Biological control is typically viewed as a two-species interaction, betweeen the agent and pest. However, there is growing recognition of the role of the broader community context on pest suppression. Over the past two decades, evidence has emerged that multiple enemy species could provide improved pest suppression, leading to improved plant growth (Snyder *et al.*, 2006). This can occur because enemies occupy different niches, for example by feeding on different pest life stages or parts of the plant (Losey and Denno, 1998), and this partitioning of the prey niche reduces interspecific competition among enemies and maximises use of the available prey.

This incorporation of predator diversity into predator-prey interactions marked an important advance, yet agroecosystems also contain multiple prey species, such that one or more pests exist within a wider food web of predators and prey. Food webs represent who eats whom in ecosystems, and thus the partitioning of prey species (the total prey niche), which can generate positive predator diversity effects, is implicit in their architecture (Thompson *et al.*, 2012). For example, in parasitoid-host food webs of forest Lepidoptera, niche partitioning (different parasitoid species tending to attack different host species) promoted overall attack rates across the community, whereas redundancy (multiple parasitoids attacking each host) tended to promote more stable attack rates (Peralta *et al.*, 2014).

In addition to understanding diversity effects on control and stability, quantitative food webs can assist with the prediction of indirect effects among species, such as when minimising non-target effects of agents on the wider community (Tylianakis and Binzer, 2014). In fact indirect effects among prey species via shared enemies underpin concepts of alternative hosts/prey outside the crop being used to augment enemy populations in conservation biocontrol. The same effect can also occur in reverse, whereby enemy populations sustained by abundant crop pests can spill over into adjacent native habitats and exert elevated predation pressure on native prey – a non-target impact (Rand *et al.*, 2006). A recent study of a Lepidoptera community and their parasitoids in adjacent native and plantation forests quantified the frequency with which each parasitoid attacks each host, to generate a quantitative food web (Fig. 2.5.1a). This could then be used to calculate the extent to which each herbivore species shared parasitoids with every other species in the community (Fig. 2.5.1a), which was used to successfully predict how changes in the

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abundance of each herbivore (e.g., via a pest outbreak) would influence attack rates on other species in the community (Frost *et al.*, 2016). These predictions worked equally well within a single forest type (native or plantation) or across different types, indicating that the landscape of different habitats functions as a single large food web in predictable ways.

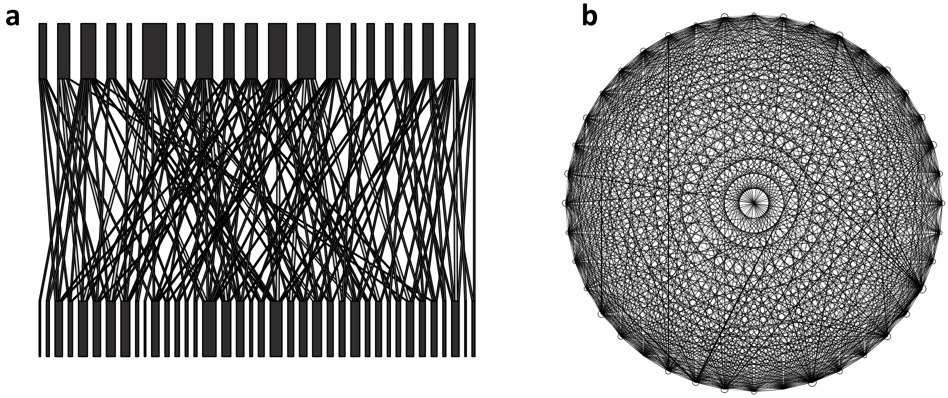

Fig. 2.5.1. A parasitoid-host food web constructed from simulated data. **a**) The top row of bars depicts parasitoid species and the bottom row represents the host species, with links between them representing feeding interactions. This is a quantitative food web, so bar and link thicknesses represent, respectively, the relative abundance of species or relative frequency of interactions. **b**) The same web can be presented as a parasitoid overlap graph. Here the circles around the periphery each represent a host species, and a link between two hosts indicates that they share at least one parasitoid species. The thickness of links indicates the relative proportion of parasitoids shared with a particular host.

Although food webs depict species as nodes, trophic interactions occur among individuals, and variation among individuals within a species may be important for determining biocontrol outcomes. For example patterns that related (i.e., genotypically similar) parasitoid individuals attack related aphid individuals (Lavandero and Tylianakis, 2013) suggest that parasitoid genotypes may have preferences for hosts, for example driven by differences among host genotypes in their immune response to parasitism (Ferrari *et al.*, 2001). A consequence of this pattern is that greater genetic diversity in the parasitoid population, combined with partitioning of host genotypes among parasitoid genotypes, may produce the same effects as those of greater species diversity and niche partitioning discussed above; namely, a fuller exploitation of the prey population.

Finally, in addition to maintaining attack rates on ecological timescales, long term sustainable biological control requires attack rates to continue on evolutionary timescales. Recent evidence that a sexual host species (the Argentine stem weevil) has acquired resistance to its asexual parasitoid (Tomasetto *et al.*, 2017) suggests that the generation of genetic diversity (via recombination) is key to evolutionary arms races among parasitoids and their hosts. In fact, evolutionary population models have indicated that the ratio of the genetic diversity of the parasitoid to that of its host is a key determinant of whether resistance will evolve (P. Casanovas, S.L. Goldson and J.M Tylianakis, unpublished results). Moreover, these models revealed that attack by multiple parasitoids could slow the evolution of resistance by the pest, suggesting evolutionary, as well as ecological, benefits of enemy diversity in biological control.

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2.6 Benefits of Pre-release Population Genetics: A Case Study Using *Psyttalia lounsburyi*, a Biocontrol Agent of the Olive Fruit Fly in California

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From a pest management perspective, limited knowledge on the genetics of released biocontrol agents have been repeatedly considered as one cause of failures in classical biological control (Hufbauer and Roderick, 2005). Introduced biocontrol agents are expected to experience a loss in genetic diversity as the result of severe bottlenecks at different stages of biocontrol programs such as foreign explorations and sampling, laboratory rearing, and field releases (Hopper *et al.,* 1993). Diversifying the geographical origins of the biocontrol agent is one way to counterbalance this effect by sampling more pre-adapted genotypes and/or producing new genotypes through hybridization (Szűcs *et al.*, 2012). In this context, conducting pre-release genetic studies to assess the genetic variation of the targeted agent at the population level is considered to be of prime importance for guiding the choice of the strains to be released (Hoddle *et al.*, 2015).

The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), was first discovered in California in 1998, rapidly spread throughout all olive growing regions, and now threatens the state's table and oil olive industries. Exploration for prospective biocontrol agents of *B. oleae* was initiated in 2000, and collections of parasitoids were most productive in sub-Saharan Africa (Daane *et al.*, 2011), which is the likely origin of *B. oleae* in cultivated olives. The most promising parasitoids were imported and evaluated in quarantines in France and USA, including *Psyttalia lounsburyi* Silvestri (Hymenoptera: Braconidae), a koinobiont endoparasitoid which is considered as a specialist of *B. oleae* (Daane *et al.*, 2011). Two *P. lounsburyi* colonies were established with parasitized *B. oleae* collected from wild olives in Kenya (Burguret Forest) in 2002, 2003, and 2005), and South Africa (SA) in 2005. A third colony was subsequently established from Kenya (Marmanet) in 2007 (Daane *et al.*, 2015). A field release permit for *P. lounsburyi* colonies of both origins was granted late 2005 and releases started in northern California in 2005 (Daane *et al.*, 2015). From 2005 to 2015, a total of 51,600 females were released (X. Wang, pers. com.). *P. lounsburyi* was recovered from field samples and is now established in coastal

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regions (Daane *et al.*, 2015), and hence one logical follow up was to identify the origin of the established populations.

The natural populations of *P. lounsburyi* from Kenya and SA that were colonized in the lab belong to two different mitochondrial lineages (Cheyppe-Buchmann *et al.*, 2011), each population harboring only two haplotypes of *CO1*. Since the onset of the mass rearing of the quarantine colonies, and over the years, a sampling of each colony was preserved for genetic analysis. The Burguret and the SA colonies were characterized by one private haplotype out of the two evidenced in each natural population by Cheyppe-Buchmann *et al.* (2011) suggesting a genetic bottleneck, and providing a simple molecular marker for identifying individuals from the mass reared colonies. In the present study, we analyzed the variation of *CO1* in one representative from each of the 25 populations recovered in California and compared them to the colonies currently mass reared at EBCL. Specimens recovered in 2015 from the field in California were preserved in ethanol and shipped to EBCL. DNA extraction, PCR and subsequent genetic analysis followed the same approach as Cheyppe-Buchmann *et al.* (2011).

We confirmed that all populations recovered in California belonged to the same species, i.e. *P. lounsburyi*. We found only two haplotypes from the populations recovered from the field; the predominant ones (20 out of 25 populations analyzed) corresponding to the haplotype of the SA colony (Fig. 2.6.1). This haplotype was widely distributed from North California (San Anselmo) to South California (Buellton), although the SA origin had been mostly introduced near San Luis Obispo (Wang, unpubl. data). Only 5 populations had the other haplotype, which matched the Burguret, Kenya, colony (Fig. 2.6.1), although this genotype had been released widely in California. In California, this haplotype was only recovered in Southern California near Santa Barbara and Ventura.

The predominance of the SA haplotype in California was quite surprising considering the fact that the SA origin represents only 3.9% of the total number of female parasitoids released (Wang, pers. com.). We assume that in the north (Bay area) the established SA populations might have resulted from the Napa/ Sonoma release while those established in the south from the San Luis Obispo release. This result also suggests that the SA population was better adapted than the Kenyan populations to the ecological conditions found in Central and Northern California. Differences in life history traits might be considered as one factor; however, it is unknown what factors may improve fitness in this region. One consequence of the present study was to shift emphasis towards mass rearing and field releasing the SA colony in California, especially in Northern California. The *CO1* mitochondrial data are being compared with data obtained from microsatellites developed at the onset of the program (Bon *et al.,* 2008) in order to determine if there has been hybridization between the two origins following establishment in California.

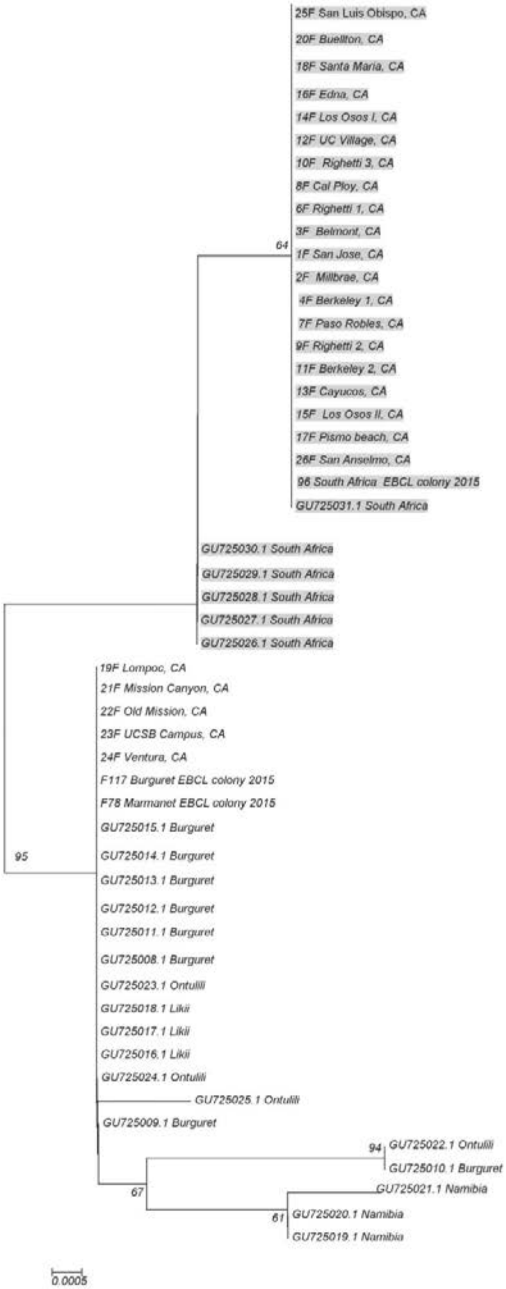

Fig. 2.6.1. Unrooted phylogenetic tree based on *CO1* (Neighbor-Joining/p-distance). Newly sequenced populations were named using the location name in California or EBCL colony Sequences from Cheyppe *et al.*, (2011) were named with their Genbank accession numbers. Numbers on nodes indicate % bootstrap support (500 replicates).

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Session 3: How Well do we Understand Non-Target Impacts in Arthropod Biological Control?

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3.1 Introduction and Non-target Effects of Insect Biological Control: Concepts, Examples, and Trends

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Whether, when, and how frequently introductions of biological control agents have important population-level effects on non-target species is a question of continuing importance to both biological control scientists and conservation biologists. Howarth (1991) first outlined an argument for significant non-target impacts of biological control agents, but this article was, in our opinion, flawed. While it opened a conversation, it did not provide a definitive answer and further discussion ensued (Follett and Duan, 2000; Follett *et al.*, 2000; van Lenteren and Loomans, 2000; Louda *et al.*, 2003; Hoddle 2004a,b,c; Stewart and New, 2007; Parry, 2009; Barratt *et al.*, 2010; Suckling and Sforza, 2014).

The impacts of these biological control agents for arthropods are less understood than those of herbivorous insects and plant pathogens released against invasive weeds. For parasitoids and predators, whose actions are generally invisible to any but specialists, we have less information on population-level impacts on non-target species. This has led to speculation that non-target impacts are high, based largely on extrapolation from several cases of likely or presumed high-level impact, especially the coccinellid beetles *Harmonia axyridis* (Pallas) and *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae) (Harmon *et al.*, 2007; Losey *et al.*, 2007) and the tachinid flies *Compsilura concinnata* (Meigen) (Boettner *et al.*, 2000) and *Bessa remota* (Aldrich) (Diptera: Tachinidae) (Kuris, 2003; Hoddle, 2006).

Past summaries of impacts of parasitoids and predators on non-target insects and mites include a mini-review for the island of Guam (Nafus, 1993); global literature reviews (Lynch and Thomas, 2000; van Lenteren *et al.*, 2006), and a detailed analysis of releases of both weed and insect biocontrol agents in Florida (Frank and McCoy, 2007). Lynch and Thomas (2000) state that nontarget effects are recorded for 1.7% of the ca 5000 recorded cases of parasitoid or predator introductions (species x country releases of about 2000 natural enemy species), as detailed in the database “BioCat” (van Lenteren *et al.*, 2006).

Non-target impacts of parasitoids and predators include (1) direct attacks on native insects, (2) negative foodweb effects, such as competition for prey, apparent competition, or displacement of native species, (3) positive foodweb effects, (4) hybridization, and (5) attacks on introduced weed biocontrol agents.

Trends (Fig. 3.1.1) showed a recent shift (2005-2015) toward a preponderance of agents with an index of genus-level (60%) or species-level (8%) specificity (with only 12% having a family-level or above index of specificity) compared to both 1985-1994 and 1995-2004,

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when 50% and 40% of introductions had family level or above specificity and only 21-27 (1985-1994, and 1995-2004, respectively) with genus, or 1-11% (1985-1994 and 1995-2004, respectively) with species level specificity. In all three decades, 11-12% of introductions could not be classified in this manner due to lack of information on natural enemy host specificity.

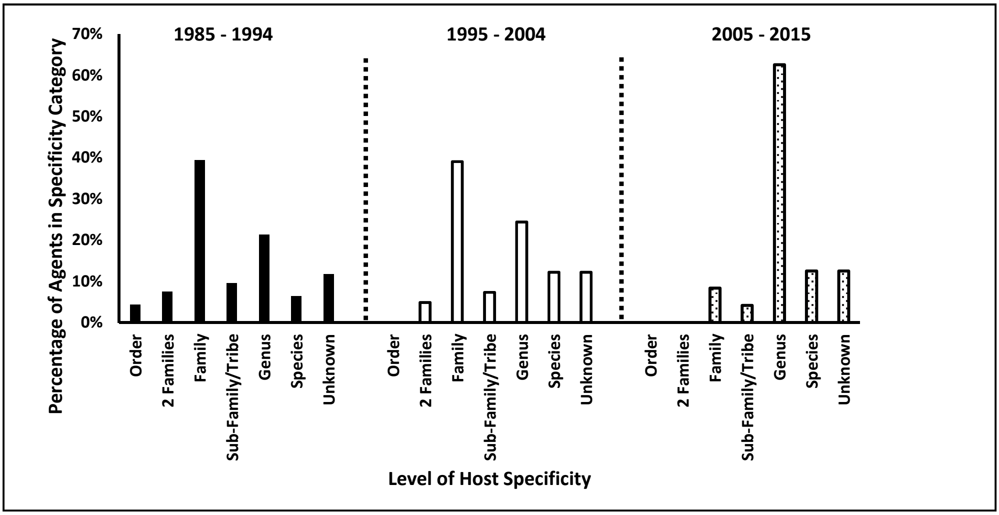

Fig. 3.1.1. Trends in levels of host specificity of parasitoids released for insect biocontrol over three decades, indicating a shift toward genus-level specificity and a reduction in use of agents with family - level specificity.

Future reductions in non-target risk from release of parasitoids and predaceous arthropods will depend on continuing to improve the forecasting of potential host ranges of agents before release. Conversely, our understanding of what impacts actually happen later under field conditions (host use that doesn't affect population densities vs. population-level impacts) will require careful study of non-target populations, with the use of lifetables, models, and experimental methods to separate impacts of co-acting sources of mortality.

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3.2 Displacement of Native Natural Enemies by Introduced Biological Control Agents in Agro-Ecosystems: A Serious Non-target Effect or Not?

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Classical biological control has been a fundamental approach to the management of invasive pests throughout the world for over 120 years. Over the last several decades there has been increasing interest and focus on examination of direct and indirect non-target impacts (Van Driesche and Hoddle, 2016). One potential indirect impact is the displacement of native natural enemies by introduced species. Invasive pest species that colonize new crop habitats are frequently attacked by a suite of native natural enemies that opportunistically take advantage of a new and abundant prey or host resource. Often, the mortality supplied by the native community is insufficient and more specific exotic natural enemies are introduced in an attempt to gain enhanced pest control. In the process, these native enemies are sometimes displaced on the target crop(s). This displacement is often interpreted as a negative non-target effect of the introduction because the native natural enemy is now found at lower abundance in the target crop(s) or is perhaps gone altogether (Bennett, 1993; Lynch and Thomas, 2000; van Lenteren *et al.,* 2006). However, this displacement also could represent the fact that the native natural enemy is now continuing to attack native hosts or prey on other host plants as they did before the invasion of the exotic pest (Van Driesche and Hoddle, 2016). The native natural enemy may now exist at lower population densities overall because their large temporary resource base has either declined (through effective biological control) or are less available because the exotic natural enemy is a superior competitor and/or more adapted to finding and exploiting hosts or prey than its native counterpart (Fig. 3.2.1). Unfortunately, the necessary data to determine if true non-target effects exist or not are often absent or weakly documented, particularly when the native enemies are naturally found in non-crop habitats.

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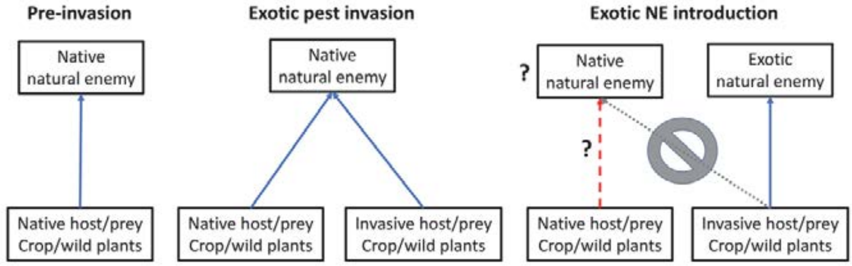

Fig. 3.2.1. Conceptual diagram of the putative displacement of a native natural enemy by an introduced natural enemy. The grey dotted line depicts displacement and the question marks ask if there is a true non-target effect (significant reduction in abundance or extirpation), or if it can now be found attacking its native hosts at approximately the same level as it did before invasion of the exotic pest.

Van Driesche and Hoddle (2016) reassessed several cases from previous reviews (Bennett, 1993; Lynch and Thomas, 2004) in which displacement of many native parasitoids was presumed to have occurred due to the introduction of exotic parasitoids, and several additional cases not reported in these earlier reviews (Table 3.2.1). In large measure, either the original assessment or the reassessments of Van Driesche and Hoddle (2016) show that non-targets have not been extirpated but essentially nothing can be said about population level impacts. Many can still be found attacking the target host to a more limited extent than before, or attacking other native insect hosts in the parasitoid’s original habitat. Universally, more work is needed to extend and expand surveys for these native parasitoids, and more importantly, studies are needed to document significant changes in population sizes of the native parasitoids relative to their former status before exotic parasitoid introductions. Such changes in abundance would be the key to understanding if non-target impacts have occurred. It is likely than many of the native species existed at relatively low densities and that populations only increased once native parasitoids began to take advantage of the abundant invasive pest species in managed habitats. The necessary studies to document any non-target effects will be extremely challenging. First, pre-release studies of the composition and abundance of the native natural enemy fauna are rare and second, resources to conduct both pre- and post-release evaluations of this nature are frequently constrained because most of the resources are focused on development and implementation of the classical biological control program.

In conclusion, the simple displacement of native parasitoid species from crop habitats after the introduction of more specialized and efficient natural enemies is not sufficient evidence to claim that a non-target impact has occurred. The assessment of the extant literature suggests that complete extirpation of a native parasitoid is rare or perhaps never happens, but more inclusive surveys and more population level studies will be needed in the systems highlighted above, and in others, before true non-target impacts can be asserted. Here only parasitoids were considered, but similar issues surround introduced insect predators such as *Harmonia axyridis* (Pallas) and *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae) where the non-target impacts of these species have been more broadly assessed (Van Driesche and Hoddle, 2016).

Table 3.2.1. Putative cases of non-target effects through displacement of native parasitoids by introduced exotic parasitoids and original assessment or reassessment of likely impacts.

Exotic agent	***Target pest***	***Non-target***	***Assessment/Reassessment of impacts***	***Location***	***Reference***
Cotesia flavipes	*Diatraea saccharalis*	*Paratheresia claripalpis* *Metagonistylum minense*	Non-targets found in some fields and in Colombia; more survey work needed	Brazil	Bennett, 1993
Cotesia flavipes	*Diatraea saccharalis*	*Apanteles diatraeae*	Non-target found in Mexico; more survey work needed in Trinidad	Trinidad, Mexico	Bennett, 1993
Aphytis holoxanthus	*Chrysomphalus aonidum*	*Pseudhomalopoda prima*	Non-target attacking native host on other plants	USA	Bennett, 1993
Aphytis holoxanthus	*Chrysomphalus aonidum*	*Aphytis costalimai*	Non-target attacking hosts on citrus in Argentina	Brazil	Bennett, 1993
Cales noaki	*Aleurothrixus floccosus*	*Encarsia margaritiventris,*	More host range testing and survey work needed on non-targets	Italy	Lynch and Thomas, 2000
Trigonospila brevifacies	*Epiphyas postvittana*	*Xanthopimpla rhopaloceros*	Non-target is not native and continues to attack target pest	New Zealand	Lynch and Thomas, 2000
Diadegma semiclausum	*Plutella xylostella*	*Diadegma mollipla,* *Oomyzus sokolowskii*	Non-targets still attacking target but at reduced rates; widely distributed and may have other hosts	Kenya	Löhra *et al.*, 2007
Eretmocerus mundus	*Bemisia tabaci*	*Eretmocerus eremicus,* *Eretmocerus joeballi*	Non-targets found attacking native hosts on other crop and urban plants	USA	Pickett *et al.*, 2013
Eretmocerus sp. (Ethiopia) ***Encarsia sophia***	*Bemisia tabaci*	*Eretmocerus eremicus,* *Encarsia meritoria,* *Encarsia luteola*	*Encarsia* non-targets found attacking target at low levels; surveys needed to document native host use	USA	Naranjo and Li, 2016

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3.3 Assessing Host Use and Population Level Impacts on Non-target Species by Introduced Natural Enemies: Can Host Range Testing Provide Insight?

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The prediction of potential non-target impacts from introduced biological control agents remains an important issue, despite significant improvements in quarantine practices. Depending on strict physiological host range, characterization in quarantine has resulted in very conservative selection of biological control agents, and no non-target impacts are known to have occurred from introductions made since 1970, at least in Hawaii (Funasaki *et al.*, 1988). Pre-release host range assessment options include investigations of host range under quarantine conditions, host range determined during quarantine programs in other areas, host ranges reported from other programs that resulted in releases, and predicted host range in the release area based on the natural enemies' host ranges in their place of origin.

This paper explores these options by review of data from pre-release screening and post-release non-target impacts, and retrospective examination of host range of parasitoids in their native provenance, compared with post-introduction non-target impacts.

Parasitism of the endemic Hawaiian koa bug, *Coleotichus blackburniae* White (Hemiptera: Scutelleridae), by prospective biocontrol agents, *Trissolcus* (Hymenoptera: Scelionidae) and *Trichopoda* (Diptera: Tachinidae) were anticipated prior to release of the insects in Hawaii. Laboratory observations suggested that the natural enemies may perform reasonably well (*Trissolcus*), to poorly (*Trichopoda*) on koa bug (Davis, 1964), although no predictions of population level impact were made. Johnson *et al.* (2005) later demonstrated that koa bug mortality from the introduced natural enemies was influenced by environmental conditions, and that accidentally introduced predators had a larger impact on the non-target host than the biocontrol agents did.

Attempts to compare predicted non-target impact using parasitism and habitat data for parasitoids from their native provenance, with actual parasitism rates in Hawaii were made, using data of known habitat and host-range from places of origin of parasitoids introduced to Hawaii (Kaufman and Wright, 2017). Results showed that if comprehensive host-range data were available from the place of origin, reasonably accurate predictions of non-target parasitism could be made using probabilistic risk assessment procedures. Results for one species, *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), are shown in Fig. 3.3.1, illustrating reasonable correlation between the predicted and actual parasitism rates.

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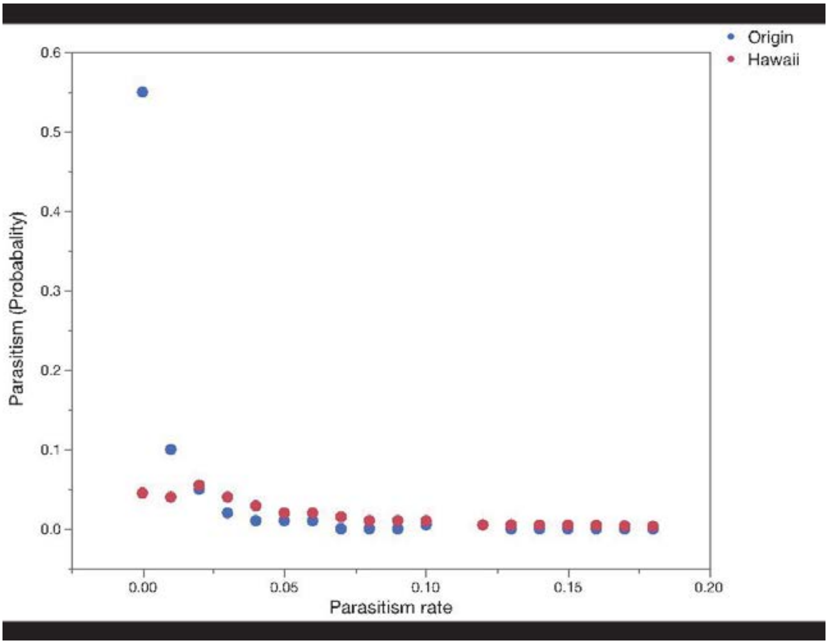

Fig. 3.3.1. Probability of occurrence of *Cotesia marginiventris* parasitism rates of an endemic Hawaiian moth, *Udea stellata*, predicted from the place of origin of the parasitoid, compared with actual levels measured in Hawaii.

Non-target host use is likely well predicted, albeit conservatively with the potential for false-positives, by physiological host range testing in quarantine. Strict adherence to quarantine testing and selecting species with high physiological host specificity appears to provide an effective protocol for avoiding non-target use. We may be able to make the process less conservative by employing probabilistic risk assessment procedures during screening, and by incorporating ecological and host-range data from previous studies. Predicting population level impacts is more complex, and requires substantial data. To predict non-target population impacts would require completion of life-table studies and careful assessment of additional stressors that may impact non-target species in the area of release.

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3.4 Parasitoid Host Ranges: Comparing Studies From the Laboratory and Field

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Laboratory assays are used to assess the host specificity of parasitoids being considered for use as importation biological control agents. The extent to which results from these assays predict the host specificity expressed in the field is an important question in biological control risk assessment. A common observation from the literatures on arthropod weed biological control agents and on entomopathogens of insects is that field host ranges are narrower than laboratory host ranges, but relatively few studies have investigated this for parasitoids (Heimpel and Mills, 2017). Here we briefly review studies comparing field and laboratory host ranges in parasitoids and then report on results from two empirical studies – one involving an aphid parasitoid that invaded North America where it is providing some control of the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), and another involving a potential biological control of a bird-parasitic nest fly that has invaded the Galapagos Islands where it is attacking Darwin's finches.

Review of the Literature: A few studies have compared laboratory and field host ranges for parasitoids used in biological control. In some of these there was evidence that these two measures of host range were quite similar (e.g., Haye *et al.*, 2005, 2015; Toepfer *et al.*, 2008; Yang *et al.*, 2008). In other cases, however, the host range found in the field was narrower than that expressed in the laboratory (Morehead and Feener, 2000; van Driesche *et al.*, 2003). In the study done by Morehead and Feener (2000), parasitoid egg injections showed that the ant parasitoid *Apocephalus paraponerae* Borgmeier (Diptera: Phoridae) could develop in nine ant species but only used one of these in the field. This suggests that suitable hosts are not always used in the field even if they are available. We know of a single case suggesting a broader field than laboratory/physiological host range, but this was due to methodological problems in which individuals of a suitable host were offered in a non-suitable condition of diapause in laboratory trials (Barratt, 2004).

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Laboratory and field host specificity in the aphid parasitoid *Aphelinus certus*: *Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae) is a parasitoid that is native to Asia but that has invaded North America on its own during or before 2015 and is now found in North American soybean fields attacking the soybean aphid (Heimpel *et al.*, 2010; Frewin *et al.*, 2010). Laboratory studies have shown that *A. certus* has a relatively broad host range, attacking and developing well in most offered species in the aphid subfamily Aphidinae (Kaser, 2016; Hopper *et al.*, 2017). Field surveys have shown that this parasitoid can indeed attack aphids other than the soybean aphid in the field, including the grain aphids *Rhopalosiphum padi* (L.) and the English grain aphid, *Sitobion avenae* (Fabricius), both of which were attacked in lab assays. In addition it was reared from the North American native aphid species *Aphis monardae* Oestlund. However, parasitism that *A. certus* displays on soybean aphids in the field in North America suggest patterns typically associated with a specialist in the sense that *A. certus* populations are tightly linked to that of soybean aphid and show strong density dependence at the per-field level (Kaser, 2016). Sampling studies suggest that *A. certus* is capable of attacking non-target hosts in the field but that its dynamics are mainly tied to soybean aphid while this aphid is dominant in North American landscapes (Kaser, 2016).

Laboratory and field host specificity in *Conura annulifera*, a parasitoid of *Philornis* flies: *Conura annulifera* (Walker) (Hymenoptera: Chalcididae) is a parasitoid known in the literature from puparia of bird-parasitic flies in the genus *Philornis* (Diptera: Muscidae). This parasitoid is native to mainland South America and is being considered as an importation biological control agent of the invasive *P. downsi* Dodge and Aitken in the Galapagos Islands (Boulton and Heimpel, 2017). Laboratory studies comparing *C. annulifera* parasitism on *P. downsi* and other fly, lepidopteran and hymenopteran species indicated that it attacked only *P. downsi* (Bulgarella *et al.*, 2017). This result was corroborated by in-field choice studies conducted in mainland Ecuador, the native range of *C. annulifera*. In these studies *C. annulifera* parasitism was compared in artificial bird nest boxes containing *P. downsi* and in experimental containers containing non-target hosts in the families Sarcophagidae and Calliphoridae. *C. annulifera* was reared only from *P. downsi* in bird nests.

Conclusions: We conclude that the results of laboratory studies of parasitoid host range tend to be upheld in field studies. Ecological filters can reduce specificity in the field to some extent but suitable hosts that coincide with parasitoids in time and space will likely be attacked in the field. Our work with *Aphelinus certus* suggests also that pest organisms that dominate the host spectrum will be attacked at a higher rate than targets and that during this time the parasitoids may exhibit population-level patterns often attributed to specialists like density-dependent responses to target host populations.

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3.5 Can Predictive Models Help to Identify the Most Appropriate Non-target Species for Host-specificity Testing?

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Selecting non-target species (NTS) for the assessment of risks posed by an entomophagous biological control agent (EBCA) can be challenging. To help with this task, we investigated the protocols that had been developed for selecting NTS previously (e.g., Van Driesche and Reardon, 2004; Kuhlmann *et al*., 2006; van Lenteren *et al*., 2006), and used them to develop a computer-based tool called PRONTI (Priority Ranking Of Non-Target Invertebrates; Todd *et al*., 2015). The aim was to develop a tool that could assess many NTS simultaneously, and, through a transparent and consistent process, prioritise them for testing with each proposed agent (Fig. 3.5.1).

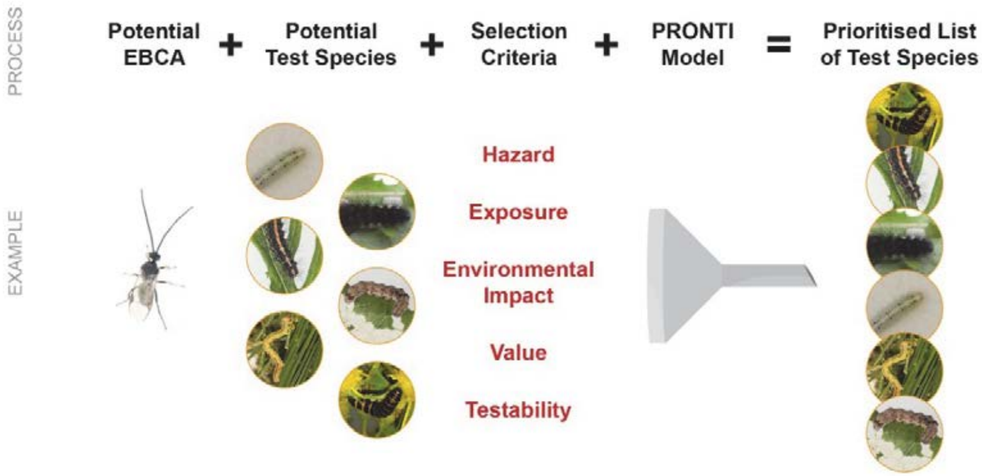

Fig. 3.5.1. The PRONTI model acts like a funnel, prioritising test species using five selection criteria. The example is for the agent, *Cotesia urabae*, released to target the lepidopteran Eucalyptus pest, *Uraba lugens*.

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PRONTI has two parts: a database of information on New Zealand's invertebrate taxa (the Eco Invertebase), and the following equation, which ranks the taxa in the database with those taxa obtaining the highest PRONTI scores prioritised for testing with the EBCA:

$$PRONTI\ SCORE = \frac{H \times E}{R} \times (S + V + T)$$

Each parameter in the equation is a score obtained by each NTS: *H* represents the hazard posed by the agent to the NTS; *E* is the potential exposure of each NTS to the agent; *R* represents the resilience of each NTS to the risks posed by the agent; *S* represents the status of the NTS in the ecosystem (i.e., biomass and food web links); *V* is the anthropocentric value of the NTS; and *T* represents the testability of the NTS.

We have used two parasitoid EBCA previously released in New Zealand to test the PRONTI tool: the Moroccan strain of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae), released in 1982 to control *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae), and *Cotesia urabae* (Austin & Allen) (Hymenoptera: Braconidae), released in 2011 to control *Uraba lugens* Walker (Lepidoptera: Nolidae) (Barratt *et al*., 2016; Todd *et al*., 2016). For each test, we compared the prioritised list of species produced by PRONTI with the list of test species that were selected using conventional methods prior to the agent's release.

For the test with *M. aethiopoides*, 82 NTS with data in the Eco Invertebase were ranked by PRONTI. Five of these species were those that had been selected for assessment with *M. aethiopoides* prior to its release in New Zealand in 1982. For this test, we used only data on *M. aethiopoides* that were available in 1982 to rank the species: data on the NTS that have been attacked by *M. aethiopoides* since its release were not included. The ten species prioritised by PRONTI are given in Table 3.5.1. Seven of the NTS in the top ten are amongst those that have been attacked by *M. aethiopoides* following its release, suggesting that, if PRONTI had been available pre-release, better predictions of non-target attack might have been made. Of the five species on the original list, only two species, *Rhinocyllus conicus* Frölich (Coleoptera: Curculionidae) and *Longitarsus jacobaeae* Waterhouse (Coleoptera: Chrysomelidae), were in the top 30 on the PRONTI list (Barratt *et al*., 2016).

For the test with *C. urabae*, we used PRONTI to rank 90 NTS for which data had been entered into the Eco Invertebase. This included the nine species originally selected for host range testing with *C. urabae* prior to its release (Berndt *et al*., 2009). The PRONTI list and the original list were very similar, with five NTS in the top nine on both lists (Todd *et al*., 2016). Post-release laboratory testing has shown that two of these species, *Tyria jacobaeae* (L.) and *Nyctemera annulata* Boisduval (Lepidoptera: Erebidae), can be parasitized by *C. urabae* (Avila *et al*., 2016), suggesting that the placement of these two species at the top of both lists was appropriate. We do not know if these species have been attacked by *C. urabae* in the field.

PRONTI has advantages, such as its ability to rank many NTS simultaneously, and its provision of a body of information that can be used to both understand each NTS ranking and to justify more objectively the selection (and rejection) of NTS for pre-release testing. It is also useful for regulatory agencies to have access to a system that is objective, consistent and reliant on published information on which to base decision-making. However, we also conclude that the time investment needed to enter data into the Eco Invertebase needs to be balanced against the objectivity provided by PRONTI when deciding which method of species selection to use.

Table 3.5.1. Top ten species ranked for testing with *Microctonus aethiopoides* using the PRONTI (Priority Ranking Of Non-Target Invertebrates) tool. The amount of uncertainty in the data used to calculate the PRONTI Score is also determined by the model. Table adapted from Barratt *et al.*, 2016. Curc = Curculionidae.

Species Name (Family: Subfamily)	Species origin	PRONTI Score	Uncertainty (%)
Nicaeana cervina (Curc: Entiminae)	Endemic	17273	8
Irenimus duplex (Curc: Entiminae)	Endemic	15445	21
Irenimus aemulator (Curc: Entiminae)	Endemic	15211	20
Irenimus stolidus (Curc: Entiminae)	Endemic	14785	16
Naupactus cervinus (Curc: Entiminae)	Self-Introduced	14431	24
Irenimus aequalis (Curc: Entiminae)	Endemic	14335	13
Irenimus albosparsus (Curc: Entiminae)	Endemic	13732	19
Irenimus egens (Curc: Entiminae)	Endemic	13584	10
Steriphus diversipes lineatus (Curc: Cyclominae)	Self-introduced	13295	28
Catoptes cuspidatus (Curc: Entiminae)	Endemic	11451	23

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3.6 What Olfactometer Tests Were Able to Tell Us About Non-target Risk That No-choice and Choice Tests Could Not

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Classical biological control of insect pests is a proven method of sustainable and cost effective pest management. However, there continue to be concerns raised about the potential risks that new biological control agents (BCAs) may pose to non-target species in the country of introduction (Follett and Duan, 2000; Eilenberg, 2006). Increasing attention is being paid to the pre-release estimation and decision making of such risk (Barratt *et al.*, 2000, 2010). Meanwhile the scientific community continues to examine how laboratory host-testing methods can best predict the ecological host ranges of proposed biological control agents (Van Driesche and Reardon, 2004; van Lenteren *et al.*, 2006).

Cotesia urabae Austin and Allen (Hymenoptera: Braconidae) is a solitary larval endoparasitoid of *Uraba lugens* Walker (Lepidoptera: Nolidae), the gum leaf skeletoniser, which is a lepidopteran pest endemic to Australia and a major defoliator of many *Eucalyptus* species (Avila *et al.*, 2013). In 2011, *Cotesia urabae* was introduced into New Zealand as a biological control agent against *Uraba lugens* (Avila *et al.*, 2013), and is now confirmed as established (Avila *et al.*, 2016a). Prior to the release of *C. urabae* in New Zealand, laboratory host-specificity testing bioassays were carried out on several non-target species (Berndt *et al.*, 2007, 2009, 2010) by conducting small arena no-choice and choice tests in containment, and following the overarching framework proposed by van Lenteren *et al.* (2006). Unfortunately high laboratory mortality for some of the more closely non-target species tested, e.g., *Nyctemera annulata* Boiduval and *Tyria jacobaeae* (L.) (Lepidoptera: Erebidae) meant uncertainty remained as to their status as potential suitable hosts of *C. urabae* (Berndt *et al.*, 2010). Also, the tests conducted measured the acceptability and physiological suitability of the non-targets being tested and did not provide much information on the habitat- and host-location process. Therefore, further assessment is needed to determine the potential risk that *C. urabae* could pose to non-target species more accurately.

Knowledge on the cues parasitoids use during habitat- and host-location are needed to better define potential ecological host range of parasitoids (Yong *et al.*, 2007). The uncertainly surrounding the potential risk that *C. urabae* was posing to non-target species, made it an ideal case study species to explore this. It has been demonstrated that *C. urabae*

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females are attracted to odours of its target host *U. lugens*, host plant (*Eucalyptus* species), and target plant-host complex (Avila *et al*., 2016b). We conducted a series of odour preference experiments in Y-tube (Fig. 3.6.1a) and four-arm (Fig. 3.6.1b) olfactometers to further investigate these attractions as well as the parasitoid's attraction towards odour cues of three non-target species, *N. annulata*, *T. jacobaeae* and *Pseudocoremia suavis* Butler (Lepidoptera: Geometridae), and their host plants (*Jacobaea vulgaris* Gaertner (Asteraceae), *Pinus radiate* D. Don (Pinaceae). These experiments aimed to (a) assess if naïve *C. urabae* females exhibit innate responses to non-target hosts, their host plants and/or non-target plant-host complexes, (b) evaluate the degree of specificity that *C. urabae* females exhibit for the target host in the presence of non-target hosts, and (c) assess potential learned responses after prior experience to non-targets.

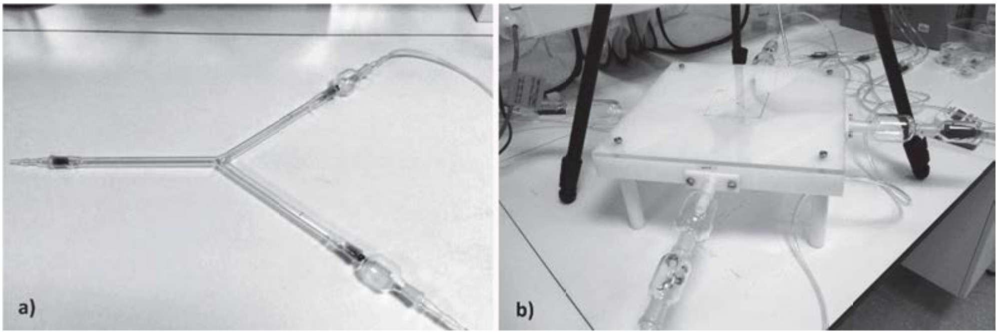

Fig. 3.6.1. Olfactometers used in our study to assess the attraction of *Cotesia urabae* towards odour cues of non-target species, their host plants, and non-target plant-host complexes: **a)** Y- tube olfactometer; **b)** four-arm olfactometer.

In Y-tube olfactometer bioassays female *C. urabae* only exhibited a significantly positive attraction to chemical cues from the non-targets *N. annulata*, and *T. jacobaeae* only, and also to their corresponding non-target plant-host complexes compared to clean air. However, no attraction occurred to the non-target host plants alone, suggesting the potential risk of *C. urabae* searching for populations of *N. annulata* and *T. jacobaeae* in the field is likely to be limited to very close range. Results from the multiple-comparison bioassays, conducted in the four-arm olfactometer, indicate that target *U. lugens* plant-host complex odours consistently produced the strongest attraction when compared with any other of the non-target (*N. annulata* and *T. jacobaeae*) plant-host complexes odours tested (Table 3.6.1). Additionally, *C. urabae* females that were given prior exposure and the opportunity to oviposit within either non-target species were not subsequently more attracted to odours of the non-targets, suggesting that associative learning is unlikely to increase non-target attack (Table 3.6.2).

Table 3.6.1. Mean (±SE) response of female *Cotesia urabae* in the exposure arena of the four-arm olfactometer, with each arm linked to odour volatiles emitted by the target plant-host complex, the non-target host complexes, and a blank control.

Response	n	**Odour field**				P-value*
		1	2	3	4	
		Uraba lugens on *Eucalyptus fastigata*	*Nyctemera annulata* on *Jacobaea vulgaris*	*Tyria jacobaeae* on *Jacobaea vulgaris*	Blank control	
No. final choices	39	27a ±0.1	9b ±0.1	3bc ±0.03	0c ±0	< 0.001
Mean no. visits/field	50	4.7a ±0.7	4b ±0.7	3.2b ±0.6	1.6c ±0.2	< 0.001
Mean % time spent/field	50	56.5a ±5.1	25.2b ±4.2	15.5b ±2.9	2.8c ±0.5	< 0.001

* P-values resulting from $\chi 2$ test (no. final choices) or Friedman two-way ANOVA by ranks. Mean within a row sharing a letter are not significantly different (P<0.05).

Table 3.6.2. Mean (±SE) response of female *Cotesia urabae*, with prior oviposition experience to non-target hosts, in the exposure arena of the four-arm olfactometer. Each arm is linked to odour volatiles emitted by the target plant-host complex, the non-target host complexes, and a blank control.

Response	Prior oviposition experience	n	**Odour field**				P-value *
			1	2	3	4	
			Uraba lugens on *Eucalyptus fastigata*	*Nyctemera annulata* on *Jacobaea vulgaris*	*Tyria jacobaeae* on *Jacobaea vulgaris*	Blank control	
No. final choices	*N. annulata*	41	24a ±0.1	11b ±0.1	6bc ± 0.1	0c ±0	<0.001
	T. jacobaeae	38	23a ±0.1	8b ±0.1	7b ±0.1	0b ±0	<0.001
Mean No. visits/field	*N. annulata*	50	4.5a ±0.4	4.4a ± 0.5	3.6b ±0.6	2c ±0.3	<0.001
	T. jacobaeae	50	4.4a ±0.6	3.8b ±0.5	3.5b ±0.6	1.7c ±0.2	<0.001
Mean % time spent/field	*N. annulata*	50	53.6a ±4.8	26.7b ±3.9	16.5b ±2.8	3.2c ±0.6	<0.001
	T. jacobaeae	50	55.3a ±5.1	24.9b ±4.2	17.1b ±3.2	2.7c ±0.5	<0.001

* P-values resulting from $\chi 2$ test (no. final choices) or Friedman two-way ANOVA by ranks. Means within a row sharing a letter are not significantly different (P<0.05).

The results from these experiments suggest that apart from a situation of overlapping distribution on a very small scale between the target host and the tested non-target hosts, *C. urabae* would retain a high degree of preference to the volatile mix corresponding to its target plant-host complex. This would then reduce the likelihood that *C. urabae* females would search for and attack *N. annulata* and *T. jacobaeae* in the field. Only if the target host is present in the immediate vicinity (blended/overlapping odours from target and non-target plant-host complexes) could we imagine that *C. urabae* may potentially encounter non-targets and, at a close range, find them attractive enough in which to oviposit.

This study demonstrates that olfactory studies could be a valuable complement to traditional laboratory host-specificity testing methods of parasitoid BCAs, as they could help to undertake more accurate assessments of the potential risks a parasitoid could pose to non-target species once introduced into a new environment. Preferably, these types of studies could be conducted within the quarantine testing period. It is likely that not all parasitoids will respond as neatly as *C. urabae* did within this type of bioassay set-up, and those species that also use visual cues for host and/or habitat location may require the use of wind-tunnels, where odour and visual cues can be presented separately, or together. But if odour volatiles are a significant cue used in host and/or habitat location, as it was in our case study with *C. urabae*, then the addition of olfactometer bioassays could be a cost-effective means of adding valuable knowledge on host specificity to the overall risk assessment of parasitoid BCAs. Therefore, we recommend that where possible, olfactory (and/or visual) response bioassays such as those reported here be introduced to quarantine laboratory host-specificity screening for potential BCAs, in order to provide useful information about the biocontrol agent's behavioural attraction to its target host complex compared to non-target species complexes. These kind of studies will bridge the knowledge gap between all the components of the host selection process, from habitat location (long-range searching) to host location (short-range searching) and then assessment of host suitability.

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Session 4: Regulation and Access and Benefit Sharing Policies Relevant for Classical Biological Control Approaches

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4.1 The New Zealand System to Assess the Environmental Benefits and Risks of Releasing New Biocontrol Agents of Arthropods

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The Environmental Protection Authority (EPA) in New Zealand regulates the importation, development and release of new organisms in New Zealand under the Hazardous Substances and New Organisms Act 1996 (HSNO Act). Under this regulatory regime, a new organism is, in part, defined as an organism belonging to a species that was not present in New Zealand before 29 July 1998, the date when the Act was promulgated. Any organism not recorded to be present in the New Zealand environment before this date is considered to be new. An EPA approval is required to import, develop, field test or release a new organism (including an exotic biocontrol agent (BCA)) in New Zealand.

Legislative criteria to obtain an approval to release a new BCA and application process: Applicants must submit a dossier of information as part of their application that provides evidence for why they consider a proposal to release a new BCA meets the regulatory requirements. This information informs the EPA and its decision makers about the risks and benefits of the prospective BCA, allows a comparative analysis of adverse and positive effects, and informs qualitative risk assessments. Furthermore, the regulator must determine whether the BCA meets a set of minimum standards in the HSNO Act.

Applicants consult with EPA staff in the preparation of an application whilst they develop a dossier. Formal processing of an application has strict statutory timeframes; a decision to release a new BCA must be made public within 100 working days of receiving a final application. Within this time the EPA invites comments from the New Zealand public and other government departments, performs a risk and cultural assessment of the application, and holds a public hearing and consideration meeting where an independent committee makes a decision whether to approve or decline the application.

Qualitative risk assessment: The HSNO Act requires a risk assessment of the effects on the environment, market economy, human health, society and communities, and Māori culture and traditions as part of our obligations under the Treaty of Waitangi. As part of a dossier, applicants must provide information about the biology of the new BCA and its taxonomic relationship with species in New Zealand, as well as information to demonstrate the likely effects on native New Zealand species and the potential economic impacts from using the new BCA. The magnitude and likelihood of each of the elements occurring is determined qualitatively, followed by an assessment of the combination of the magnitude and likelihood to assign a level to and result of the effect, i.e. negligible, low, medium or high. We weigh combined benefits against combined risks to determine whether the

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benefits outweigh the risks, or vice versa. In some instances, qualitative risk descriptors over-simplify the trade-offs between environmental risks and economic benefits, and the EPA avoids using a risk matrix.

Here we discuss two previous applications for invertebrate BCAs to demonstrate the EPA's approach to risk assessment. Both agents, *Tamarixia triozae* Burks (Hymenoptera: Eulophidae) and *Macrolophus pygmaeus* Rambur (Heteroptera: Miridae), could establish self-sustaining populations in the environment and were being considered for classical biological control use, although *M. pygmaeus* was intended for release in greenhouses.

Risk assessment assumptions: The EPA risk assessments assume that a BCA will successfully establish in the New Zealand environment and develop self-sustaining populations. If a BCA establishes a large population, the frequency of potential risks occurring increases and, at the same time, the benefits will also increase. Therefore, an assessment made on full establishment of a BCA in its introduced range makes it easier to weight benefits against risks.

Risks and benefits: The EPA took into consideration results from host range testing that demonstrated *T. triozae* as a BCA for tomato potato psyllid, *Bactericera cockerelli* Sulc (Hemiptera: Triozidae), a pest of crop plants in the solanaceous family, would not form self-sustaining populations on any species that were tested other than *B. cockerelli*. However, native species, *Trioza panacis* Maskell (Hemiptera: Triozidae), is within its physiological host range and *Trioza curta* Ferris & Klyver may die if *T. triozae* encounters and attacks this psyllid. They are also rare and threatened psyllids in New Zealand for which limited information is available. The EPA risk assessment included contemporary scientific knowledge regarding parasitoid foraging behaviour, the occurrence of environmental refuges and sequential steps that would have to occur for *T. triozae* to reach and parasitize rare psyllids. The EPA assessed whether *T. triozae* would operate in cropping environments where high densities of *B. cockerelli*'s host plants are found, and considered that it is unlikely to be attracted into native habitats due to its olfactory responses to host plants based on research that was undertaken on another *Tamarixia*-host system; whether *T. triozae* would employ chemical cues to forage for *B. cockerelli* in the immediate environment where its food plants are present; whether native psyllids are found in crop systems; and potential overlap between the pest's non-crop food plants and host plants for native psyllids in crop-border areas, creating refuges for native psyllids. The magnitude of adverse effects and likelihood of any effects occurring was considered separately for native psyllids in crop and border systems, native psyllids in native habitats, and rare or threatened psyllids.

In our second example, the weighing of potential positive and adverse effects from the release of *M. pygmaeus* in greenhouses was measured across individual, local and regional scales. The EPA assessment found that the risks to the environment in the immediate vicinity of glasshouses are negligible, while human health benefits to be gained through reduced organophosphate applications are likely. The benefits therefore outweighed risk at this scale. The risks to the modified environment surrounding glasshouses and benefits to the economy were assessed to be non-negligible, and the benefits were found to likely outweigh risks at the local scale. Finally, the risks to the environment at a regional to national scale were assessed to be non-negligible, and the decision makers considered that the benefits at this scale were unquantifiable.

The benefits of *T. triozae* to control tomato potato psyllid rested on independent economic analysis of the predicted reductions in costs to control *B. cockerelli* that might be generated from this BCA (Fig. 4.1.1). Further, the release of *T. triozae* would have beneficial effects on the environment by reducing broad-spectrum insecticide use and re-establishing integrated pest management in *B. cockerelli* affected crops. The EPA assessed that the BCA will help growers of traditional Māori solanaceous crops to control *B. cockerelli*, allowing Māori and other amenity gardeners to return to traditional and organic control methods.

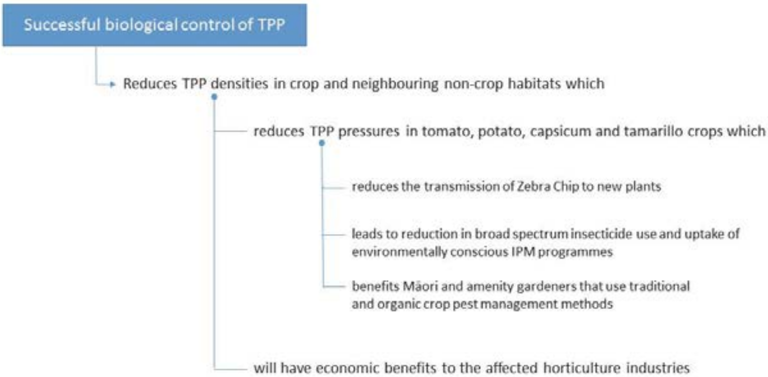

Fig. 4.1.1. EPA assessment pathway to demonstrate positive effects that may follow release of *T. triozae* for control of *B. cockerelli* or TPP (the tomato potato psyllid). Zebra chip is a disease transmitted by *B. cockerelli* – the causal agent is the bacterium *Candidatus Liberibacter solanacearum*.

Concluding remarks: The New Zealand system to assess new BCAs for arthropods is based on scientific data, economic analysis, contemporary evidence and cultural and broader societal impacts. It is a time-bound and transparent process. The EPA increasingly includes post-release validation of predictions from quarantine testing or field evaluations in its assessments. In addition, new qualitative and belief modelling approaches that rely on scientific information and expert elicitation may be used to improve risk predictions and reduce uncertainty in decision making in the future, enhancing the EPA's challenging task of weighing complex benefits against complex risks.

4.2 Practical and Implementable Mechanisms for Compliance with the Nagoya Protocol: Access and Benefit Sharing

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Live cultures for use in the development of basic and applied science must be collected and utilised in compliance with the regulatory environment. In October 2014 the Nagoya Protocol on Access and Benefit Sharing (ABS) came into force and its implementation is the responsibility of all countries that are signatory to the Protocol. By June 2017 there were 96 Parties to the Nagoya Protocol and 3 had ratified and still to become a party; of these 31 countries had published legislative, administrative or policy measures. Many countries in Europe, such as the UK, have chosen not to put access controls in place at this time, but others already have laws controlling access, see the ABS Clearing House (2017).

In its work, CABI accesses biological and genetic resources and undertakes sampling and collection of biological materials including, among other uses, for discovery of biological control agents to manage invasive species. CABI is working with its partners to establish best practices to comply with ABS requirements. CABI is an international not-for-profit organization that improves people's lives worldwide by providing information and applying scientific expertise to solve problems in agriculture and the environment. CABI's 48 member countries guide and influence its work which is delivered by scientific staff based in a global network of centres (CABI, 2017). Thirty of the 48 CABI member countries (Fig. 4.2.1) have already taken steps to implement ABS measures, 9 have ratified, 8 signed with 13 Party to the Protocol. Several member countries have provided useful feedback resulting in the first agreement with a provider country, Ghana, being signed.

Fig. 4.2.1. CABI member countries.

European Union Regulation no. 511/2014 implements the Nagoya Protocol elements that govern compliance measures for users. It offers the opportunity for users of genetic resources to demonstrate due diligence in sourcing their organisms by selecting from holdings of ‘registered collections’. The UK has introduced a Statutory Instrument, the Nagoya Protocol (Compliance) Regulations 2015 (UK, 2017) which puts in place enforcement measures within the UK to implement this Regulation. The objective of the Protocol is to ensure benefit sharing from the utilization of genetic resources and associated traditional knowledge in order to contribute to the conservation and sustainable use of biodiversity. The goal is to prevent the utilization of genetic resources, or associated traditional knowledge, which were not accessed in accordance with the national access and benefit-sharing legislation or regulatory requirements of a Party to the Nagoya Protocol. Therefore users of genetic resources need to be aware of the requirements of both the country in which they work and those of the provider country. These differ from country to country. Importantly the Protocol has been developed to support the effective implementation of benefit sharing commitments set out in mutually agreed terms (MAT) and to improve legal certainty in utilization of genetic resources and traditional knowledge.

To facilitate the process of compliance, scientific communities have begun to design policy and best practices; a number of are available via the ABS Clearing House (2017). The Microbial Resource Research Infrastructure (MIRRI), a pan-European distributed research infrastructure that provides access to high-quality micro-organisms for research, development and application has published the MIRRI Best Practice Manual on ABS (Verkley *et al.*, 2016). This provides guidance for microbial domain Biological Resource Centres (mBRCs) in implementing their ABS policies and covers the acquisition of material, supply to third parties and the delivery of other services. It increases transparency on how mBRCs conduct research on their holdings. The European Commission has published general guidance on the scope of the EU Regulation (2017) and is currently drafting sector specific guidance; of specific relevance is the *Guidance Document for the Biocontrol and Biostimulants sector*. The main purpose of this document is to arrive at a shared interpretation of the terms “utilisation” and “research and development” as contained in Regulation (EU) No 511/2014. It enables the user to determine what activities are in and out of scope of the EU regulation. It explores the range of activities that may be carried out in the context of product development, and presents the obligations of users that follow.

The EU regulation requires a user of genetic resources or the associated traditional knowledge to declare that benefit sharing mechanisms are in place when securing funding for the research and secondly when a product goes to market. It is applicable when conducting research and development on the genetic and/or biochemical composition of genetic resources, including through the application of biotechnology. The EU Regulation applies:

- to genetic resources from countries that exercise sovereign rights
- to genetic resources when countries have established applicable access measures and ratified the Nagoya Protocol
- if genetic resources were accessed after 12 October 2014
- to genetic resources that are not already governed by specialised international instruments

In the EC guidance document several case scenarios are described. The isolation, growth and storage do not constitute utilisation in the context of the EU Regulation. However, activity that selects and optimises the biochemical properties of a genetic resource is considered 'utilisation'. If the taxonomic characterization of a genetic resource is combined with the discovery of specific genetic and/or biochemical properties, this would again qualify as 'utilisation'. Testing of a new product based on genetic resources for regulatory requirements does not constitute research and development. However, if based on the outcome of the tests, further research and development is carried out on the genetic and/or biochemical composition of genetic resources on which the product is based, in order to fulfil additional requirements, then this is within the scope of the EU ABS Regulation.

The International Organisation for Biological Control (IOBC) has produced specific best practice for the use and exchange of biological control genetic resources (IOBC, 2009; Cock *et al.*, 2010). More recently, Mason *et al.* (2017) proposed best practice which includes collaborations to facilitate information exchange, knowledge sharing, cooperative research to develop capacity in source countries and transfer of production technology to provide opportunities for small-scale economic activity. There are a number of publications that raise awareness and offer specific guidance for example its implication for microbiology (Smith *et al.*, 2017) and more generally (Beckett, 2017). However, the key source of information is the ABS Clearing House (https://absch.cbd.int/), a platform for exchanging information on access and benefit-sharing where each Party to the Nagoya Protocol is required to make available legislation, contacts for the national focal point and competent national authority and permits issued including PIC – Prior Informed Consent, MAT – Mutually Agreed Terms and the IRCC – Internationally Recognised Certificate of Compliance.

The CABI Development Fund supported a project to introduce ABS best practice producing an information resource to keep CABI staff aware of developments and assist their compliance and a strategy to reduce administrative burden for both provider and user. CABI is currently seeking approval of policy and procedure from National Authorities; negotiating open access for its scientists to collect materials through a single agreement using the genetic materials solely to deliver its mission. A description of all uses CABI staff make of genetic resources and a list of benefits that CABI provides in return for access, alongside its best practice, define the terms and conditions for negotiation. If commercial use is envisaged or is serendipitously discovered, this will constitute a new use and CABI will negotiate appropriate benefit sharing specifically for this. This process is beginning to bear fruit with Ghana a signatory to a memorandum of understanding (MoU). CABI envisages at least four levels of country response:

1. A country may see no reason to agree anything if they not claiming sovereign rights and controlling access to their genetic resources
2. In Europe the majority of countries are not claiming access but want users to employ "due diligence"; CABI is seeking country endorsement or agreement its best practice in such cases
3. Countries who wish to claim sovereign rights and control access may want something more formal and this could be a two levels the MoU or:
4. A more formal contractual agreement (a rough first draft is available)

It is hoped that when most countries have become Party to the Nagoya Protocol access and use of genetic resources will become easier as providers will know that users will be obliged to implement benefit sharing commitments.

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4.3 Access and Benefit Sharing: Best Practices for the Use and Exchange of Invertebrate Biological Control Agents

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The Convention on Biological Diversity (CBD) acknowledges the sovereign rights that countries have over their 'genetic resources'. The Nagoya Protocol that came into force in 2014 provides a framework for the implementation of a fair and equitable process by which access to genetic resources, and sharing of benefits from use between donor and recipient countries can take place. Biological control agents are genetic resources and countries which are signatories to the Protocol must develop a legal framework to ensure that compliant procedures are in place both to secure access to organisms from the donor country, and to share benefits arising from their use in the recipient country.

The International Organisation for Biological Control (IOBC) Global Commission on Biological Control and Access and Benefit Sharing strongly recommended that biological control agents should be considered as a special case with respect to access and benefit sharing (ABS) under the CBD (Cock *et al.*, 2010). Recently, IOBC has prepared a best practices guide to assist the biological control community to demonstrate due diligence in complying with ABS requirements (Mason *et al.*, 2017). The guide proposes that best practise should, where possible, include the aspects described below.

Collaborations to facilitate information exchange about what invertebrate biological control agents are available and where they may be obtained: Biocontrol practitioners have, over many years, developed an international 'community of practice' based on free and collegial exchange of biological control agents. Information on successful use of biological control agents is often not published and so informal communication between researchers has enabled access to knowledge of availability and efficacy of agents used elsewhere. An example of this, although not formalised under ABS protocol, is a recent

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collaboration where the United States Department of Agriculture - Agriculture Research Service provided information and specimens of the egg parasitoid, *Trissolcus japonicus* (Ashmead) to New Zealand where they have been used in pre-emptive risk assessment quarantine studies in advance, and in preparation for the possible incursion of the invasive brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae). Under the Nagoya Protocol, such collaborations may need to be formalised and subject to contractual agreements. We recommend that agreements be developed in such a way that they will clearly benefit the source country by providing opportunities for capability building, joint research programmes, and joint publications.

Sharing of knowledge through freely available databases that document successes and failures: Compilations have been made of the introduction and impact of classical biological control (CBA) agents. Mic Julien's world catalogue of the use of CBAs for weeds (Winston *et al.*, 2015) provides comprehensive coverage. Hajek *et al.* (2005) catalogued the pathogens and nematodes used for CBA of insects and mites; an updated catalogue is in press and will be available on-line (A.E. Hajek, pers. comm. 2017). Cock *et al.* (2016) updated the BIOCAT database of introductions of insect CBAs to control insects to include information from publications to the end of 2010. Work is planned to make this database available very soon in a searchable format via the Plantwise knowledge bank (http://www.plantwise.org/KnowledgeBank/home.aspx). Thereafter, CABI plans to further update BIOCAT, and broaden the scope to the use of all CBAs to control invertebrates.

For augmentative biological control, van Lenteren *et al.* (2017) have compiled information about natural enemies and microorganisms available for pest and disease control worldwide; this publication is open access and thus widely accessible. Both biocontrol practitioners from developing and developed countries have collaborated in many augmentative biological control projects and in the compilation of the information published in the above mentioned paper.

Cooperative research to develop capacity in source countries: An example involves ongoing international research to explore options for biological control of an invasive bird-parasitic fly in the Galapagos Island that is threatening Darwin's finches with extinction (Boulton and Heimpel, 2017). This project builds upon a recent success in biological control of cottony cushion scale in Galapagos (Hoddle *et al.*, 2013) and involves universities in the USA, Argentina and Trinidad and Tobago as well as the Charles Darwin Center and the Galapagos National Park Service, both in Ecuador. The project has led to research collaboration and co-authorship among these and other institutions (Bulgarella *et al.*, 2015, 2017; Delvare *et al.*, 2017) and capacity building via construction of a new laboratory and renovation of a quarantine laboratory at the Charles Darwin Research Station in Galapagos. It has launched the careers of one Mexican and two Ecuadoran students, who have moved from this project to graduate degree programs in Chile, Austria and the USA. This ongoing project will likely continue to build capacity for biological control in Ecuador and provide valuable training for Latin American students.

A second example involves collaboration in an ongoing biocontrol program for invasive spotted wing drosophila. US scientists at the University of California at Berkeley and the USDA-ARS, along with Italian scientists at the Consiglio Nazionale della Richerche, are working with Chinese cooperators at the Yunnan Provincial Academy of Agricultural Sciences. The Chinese have been integrally involved in host surveys and exploration for natural enemies in the province. The US and Italian scientists have provided the Academy staff with training during exploration visits to China, and have hosted their visits to laboratories in the USA for additional training which is expected to lead to a graduate degree in China.

In the biocontrol industry cooperative projects have been set up between individual companies and research institutes in provider countries, where local research institutes were involved in collection and screening of potential natural enemies. The institutes benefit from exchange of knowledge on culturing organisms and the development of solid screening protocols. For example, Koppert B.V. has collaborated closely with the University of Sao Paulo in Brazil to collect, identify and evaluate phytoseiid predatory mites. The work was carried out by Brazilian students supervised by Koppert at their R&D department in the Netherlands, and to date has led to a successful PhD study.

Transfer of production technology to provide opportunities for small-scale economic activity: During the past 50 years, when augmentative biological control has become increasingly adopted, members of IOBC have often assisted in and provided information for setting up mass rearing programmes for natural enemies and microbial organisms in developing countries. Also IOBC members have organised courses on setting up biocontrol projects, proper mass rearing techniques and quality control procedures for biocontrol agents.

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Session 5: The Role of Native and Alien Natural Enemy Diversity in Biological Control

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5.1 Native Coccinellids and Biological Control: A Positive Partnership that can be Threatened by the Invasion of an Alien Species

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Biodiversity is threatened (http://www.livingplanetindex.org/home/index) and the major factors responsible for the decrease in biodiversity are land use change, especially the conversion from natural habitats to agriculture, and biological invasions, which can act synergistically (Didham *et al.*, 2007; Newbold *et al.*, 2015). Nevertheless, agroecosystems can support some biodiversity (Norris, 2008; Grez *et al.*, 2013). There is a strong consensus that biodiversity is usually positively related with ecosystem functioning, including biological control, via complementarity and sampling effects, as proposed by the insurance hypothesis (Yachi and Loreau, 1999; Loreau *et al.*, 2003; Shanafelt *et al.*, 2015). Within this biodiversity, native natural enemies may play an important role in pest control in agroecosystems, although their effects in comparison to exotic species have been scarcely explored in the literature. In this work, we will present an example of how native coccinellids play an important role in biological control, and how this service can be threatened by an invasive alien species.

Coccinellids are important predators of aphids and can be effective in reducing within-season densities of some species of aphid pests (Obrycki *et al.*, 2009). In alfalfa fields in central Chile, coccinellid assemblages used to be very diverse and mostly dominated by native species, especially *Eriopis chilensis* Hofmann (Coleoptera: Coccinellidae). For example, in the 2002-2003 season, more than 80 % of the 8,500 coccinellids sampled were native and from those 75% were *E. chilensis* (Zaviezo *et al.*, 2006). In field experiments with sentinel aphids, *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) in alfalfa, coccinellids, specially *E. chilensis*, accounted for most of aphid predation, highlighting their importance as biocontrol agents (Ximenez-Embun *et al.*, 2014). Also, in cage experiments carried out to assess biological control of *A. pisum* in alfalfa fields, native coccinellids were positively associated with biological control, but not alien coccinellids (Grez *et al.*, 2014). Therefore, native natural enemies may play a crucial role in controlling pests and thus they should be conserved within agroecosystems.

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Native to Asia, the harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), has invaded many regions of the word, where negative effects on native natural enemies have been observed (Roy *et al*., 2016). Its recent invasion into Chile has also resulted in a decline in the diversity of coccinellids and in the abundance of native coccinellids in alfalfa fields, possibly due to antagonistic interactions like intraguild predation (IGP) or competition (Grez *et al.,* 2016). *Harmonia axyridis* is today the most abundant coccinellid in alfalfa fileds in central Chile in early spring.

In alfalfa fields we have studied IGP on common coccinellids in this crop: the native *E. chilensis* and the aliens *H. axyridis* and *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae). Experiments with sentinel eggs have shown that *E. chilensis* and *H. variegata* are predated on more than *H. axyridis* (52%, 59% vs 35%). Also, molecular gut content analysis indicated that *H. axyridis* is the more frequent intraguild predator (50% of all intraguild predation events), and that most of its predation is on *E. chilensis* (64%). These results point out that there is an asymmetry in IGP among these species, with *H. axyridis* being the strongest intraguild predator.

In the laboratory we have studied voracity (daily aphid consumption) and competition (proportional weight gain) between these species under limited prey availability. *Harmonia axyridis* was twice as voracious as the other two species. Weight gain, in the absence of competitors, was similar for all three species, but in heterospecific combinations *H. axyridis* gained more weigth than the other two. Thus, *H. axyridis* seems to be a better competitor.

Because of assymetric intraguild predation and competition in favor of *H. axyridis*, the positive partnership between native coccinellids and biological control observed previous to the arrival of this invasive species could be disrupted. On the other hand, because of its higher voracity, *H. axyridis* may be a good biocontrol agent, but only if it can compensate for the loss of service provided by native coccinellids througout the season, something that needs to be assessed.

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5.2 Predator Invasion Disrupts the Conservation of Natural Enemy Biodiversity

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Pest suppression may be strongest when natural enemy communities are species rich (high richness) with similar abundances among those species (high evenness). While conservation biological control has focused on encouraging greater species richness, it is less clear how to promote greater natural enemy evenness. Working on over 50 mixed-vegetable farms across three US states, we used structural equation models to examine relationships between evenness of ground-active generalist predators and the availability of two resources – non-pest prey and refuges habitats – thought to encourage greater natural enemy biodiversity. We found that higher densities of detritus-feeding springtails, important non-pest prey, correlated with increasing predator evenness. However, increasing densities of the invasive ground beetle *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae) correlated with reduced evenness among native predators, counterbalancing any benefit of springtails. Structural complexity and alternative food provided by non-crop plants encouraged higher densities of *P. melanarius*, reinforcing the harm of the invaders to predator evenness. Altogether, these results suggest that the presence of a non-native ground beetle complicates any effort to conserve evenness among native ground-foraging natural enemies. Indeed, negative effects of *P. melanarius* appeared sufficiently strong to overwhelm efforts to promote greater predator evenness by enhancing prey diversity, food plants, or shelter.

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5.3 Impacts of North American Native and Introduced Natural Enemies on Population Dynamics of the Invasive Emerald Ash Borer

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The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), an invasive phloem-feeding beetle from Asia that attacks ash (*Fraxinus*) trees, was inadvertently introduced to North America during the 1990's. EAB was first detected in Michigan, USA and Ontario, Canada in 2002 and has since spread through much of eastern and central North America, where it has killed hundreds of millions ash trees.

Five years after the discovery of EAB in North America, a classical biological control program began with the introduction of EAB parasitoid species from Asia (Bauer *et al.*, 2015). To date, several EAB biocontrol agents have been approved for release: two larval parasitoids, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) and *Spathius agrili* Yang (Hymenoptera: Braconidae) and one egg parasitoid, *Oobius agrili* Zhang & Huang (Encyrtidae), from northeast China (Federal Register, 2007). More recently, a fourth EAB parasitoid, *Spathius galinae* Belokobylskij & Strazanac (Hymenoptera: Braconidae) from the Russian Far East, was also approved for release (Federal Register, 2015). Environmental releases of the three Chinese parasitoid species began in Michigan in 2007 and the Russian parasitoid in 2015, with small numbers of each species released initially. However, larger releases began in 2010 when the USDA APHIS mass-rearing facility was established in Brighton, Michigan (Bauer *et al.*, 2015; Duan *et al.*, 2015, 2017). By the summer of 2016, 24 EAB-infested states and two Canadian provinces had released one or more species of these biocontrol agents (MapBiocontrol, 2016).

In 2008, following the first releases of these biocontrol agents, we began studying the stage-specific mortality of immature EAB including that caused by the introduced biocontrol agents, native natural enemies, and other biotic factors. The study sites are located in six, southern Michigan hardwood forests near the epicenter of the EAB invasion, and the parasitoid introductions occurred near peak EAB densities prior to widespread ash tree mortality. The data were collected each year from 2008 to 2016, and partial life tables of EAB were constructed for each study site each year and analyzed to determine the role of North American native natural enemies and introduced biocontrol agents in suppressing populations of immature EAB life stages (Duan *et al.*, 2015).

Results of our study revealed that EAB populations were heavily attacked by woodpeckers, undetermined biotic factors (such as pathogens and/or host plant resistance), and a diverse group of native hymenopteran parasitoids (primarily species of *Atanycolus*

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(Hymenoptera: Braconidae)) during the earlier phase of the study (2008- 2012), and then by *T. planipennisi*, one of the introduced biocontrol agents from China in the later phase of the study (2012-2016). Parasitism by both the North American native parasitoids and the introduced larval parasitoid contributed significantly to the reduction of net EAB population growth rates in our study sites from 2010 to 2016.

Life-table analyses of EAB populations further indicated that North American native, generalist natural enemies (primarily *Atanycolus* spp.) played a significant role in reduction of EAB population density during the outbreak phase of the invasion, when the introduced natural enemies were just being released and had not yet established large enough populations to provide significant suppression of the target pest. However, *T. planipennisi* established a self-sustaining population approximately 4-5 years following its release, spread to other infested areas, and provided significant suppression of EAB population growth in the aftermath of EAB invasion. Together, the North American native natural enemies and the introduced agent are providing significant biocontrol services against EAB, allowing ash seedlings, sapling, and young trees to fill forest gaps thereby facilitating the gradual recovery of forested ecosystems in southern Michigan.

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5.4 Relationships Between Diversity of Natural Enemy Communities and Pest Predation Levels in Different Farming and Landscape Contexts in Hedgerow Network Landscapes

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Biological control of pests by their natural enemies is considered as a key ecological process to reduce pesticide use in modern agricultural systems. A problematic issue in actual research on biological control is the absence of a consensus regarding the relationships between biodiversity of natural enemies and levels of pest control (Loreau *et al.*, 2001). While some studies have shown the importance of predator diversity, in terms of species richness, abundance or functional diversity (based on ecological traits such as body size) (Snyder *et al.*, 2006; Rudolf *et al.*, 2014), others have highlighted the role of predator species identity in pest predation levels (Cardinale *et al.*, 2003; Straub and Snyder, 2006). One major difficulty lies in relating results from small-scale experimental approaches where only a few predator species are manipulated, and the effective diversity of natural enemies and levels of pest controls in "real" landscapes at large spatial scales (Kremen, 2005; Tschnartke *et al.*, 2007). Another crucial issue is to determine the key environmental factors that drive predator biodiversity and pest predation processes themselves. Most studies have investigated either the effect of local management or the effect of landscape heterogeneity. Existing literature reports the positive influence on pest predation levels of low input farming practices (especially organic farming) at the field or farm scale (Bengtsson *et al.*, 2005), and of spatial landscape heterogeneity related to the amount of semi-natural habitats (SNH) (Bianchi *et al.*, 2006). More recent studies have also explored the role of farming system heterogeneity (mainly organic vs. conventional farming) at the landscape scale, but this has led to contradictory results (e.g., Gabriel *et al.*, 2010; Puech *et al.*, 2015). As the knowledge on the effects of local and landscape factors is fragmentary and controversial, it appears important to disentangle their effects in order to identify key management options likely to enhance biological control. In the present study, we investigated the relationships between communities of natural predatory arthropods and pest predation levels, considering different contexts in terms of local (field scale) farming systems (organic OF vs. conventional farming CF) and landscape heterogeneity related to both SNH and farming systems. We addressed this issue by considering communities of

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carabid beetles, which are considered major predators of various crop pests in many agricultural systems (Kromp, 1999).

A survey of carabid communities and predation levels of sentinel prey (*Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) and weed seeds (*Viola arvensis* Murray (Violaceae) was conducted in 2016 in 20 pairs of OF and CF winter cereal fields in Brittany, Northwestern France. Fields were distributed along a landscape gradient with varying percentages of OF (1-40%) and SNH (1-20%) in 500 m radius circles around sampled fields (Puech *et al.*, 2015). Landscape heterogeneity related to SNH, land-uses, and farming systems (OF and CF) was characterized by composition and configuration metrics within 125, 250, and 500 m radius circles centered on each field. Three components of carabid communities were considered to analyze community-predation relationships: total species diversity (activity-density and species richness), functional diversity (activity-density and species richness of groups based on body size or diet), and species identity (activity-density of the 6 dominant species).

Our results showed that pest predation levels were poorly related to any components of carabid communities. Neither species or functional diversity of carabids, nor activity-density of the dominant species did significantly influence predation rates of aphids or weed seeds, except for the carabid species *Brachinus sclopeta* (Fabricius) (Coleoptera: Carabidae). Although carabid beetles are highlighted as major natural control agents of pests in various agricultural systems worldwide (Kromp, 1999), our study suggests that predation processes in our landscapes might be related to more complex communities involving other guilds of predators. Our study also brings insights about the drivers of predation processes in relationships with farming and landscape contexts of crops (Table 5.4.1).

Table 5.4.1. Average effects (multimodel inference with mixed generalized linear models) of local farming type (Farming OF: organic farming / farming CF: conventional farming), landscape heterogeneity (% of grassland, % of OF or CF, and land-use diversity within 125, 250, or 500 m radius circles) and interactions between local and landscape factors. Only significant variables are shown.

Significant variables		**Estimate**	**Standard error**	***P*-value**
Aphid predation	Farming CF:Land-use diversity (125 m)	0.229	0.091	0.011
Seed predation	Land-use diversity (125 m)	-0.075	0.031	0.015
	Farming CF:% grassland (125 m)	-0.186	0.058	0.001
	% CF (125 m)	-0.065	0.026	0.014
	Farming CF:% OF (125 m)	-0.129	0.056	0.021
	Farming CF:% CF (125 m)	0.098	0.044	0.026
	Farming CF:% grassland (250 m)	-0.092	0.037	0.012

Prey predation rates were similar in OF and CF fields. This might be related to higher overall biodiversity in OF fields, which could either result in negative interactions between predator species (competition, intraguild predation), or in higher availability of alternative prey to the detriment of sentinel prey consumption. At the landscape scale, predation of aphids and seeds were related to land-use diversity (Shannon index) in the surroundings of cereal fields (125 m). In the case of seeds, predation rates were further explained by interactions between local farming type (CF) and (i) the percentage of grassland (125 m, 250 m) and (ii) percentage of OF or CF in the field surroundings (125 m) (Table 5.4.1).

To conclude, our study highlights the difficulty of relating prey predation levels with "real" species-rich and complex communities of natural enemies at spatial scales larger than experimental ones. Realizing extensive surveys of biological control at large spatial scales is nevertheless important to better explain the variability in natural predation processes in relationships with farming and landscape contexts. Further investigation is thus needed to better understand the interactions between the complex structure of predator metacommunities and pest predation levels.

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5.5 Establishment of *Mastrus ridens* (Hymenoptera: Ichneumonidae), an Ecto-parasitoid of Codling Moth, in New Zealand

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Mastrus ridens Horstmann (Hymenoptera: Ichneumonidae: Cryptinae), originally described as *Mastrus ridibundus* (Horstmann, 2009), is a synovigenic gregarious, idiobiont ectoparasitoid of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). It was collected in Kazakhstan (as *M. ridibundus*) in the 1990s and released in California, Argentina and Chile to control codling moth (Hougardy and Mills, 2006). Following approval from New Zealand's regulatory agency, *M. ridens* was imported into New Zealand from Argentina in January 2009. After an extensive host testing programme (Charles *et al.*, 2013a,b) *M. ridens* was first released in Hawke's Bay in October 2012.

Laboratory cultures of both codling moth and *M. ridens* were maintained in Auckland. Diapausing codling moth larvae were reared on artificial diet (Singh and Ashby, 1986). Mature codling moth larvae were manually removed from rearing tubes and provided with cocooning sites within a 2- to 3-cm wide strip of corrugated cardboard rolled into a c. 8-cm diameter roll. Fifty codling moth larvae were allocated to each roll. Diapausing larvae produced in this way were stored at 4°C for rearing parasitoids.

A colony of *M. ridens* was maintained at 22±1°C, by exposing rolls of diapausing codling moth for parasitism (Sandanayaka *et al.*, 2011) to produce parasitoids for colony maintenance and field releases. We developed a system that efficiently produces a regular supply of parasitised, cocooned codling moth larvae, which are stored at 4-5°C. This allows us to increase *M. ridens* numbers rapidly to produce large numbers for releases.

Release of *Mastrus ridens*: Approximately 230,000 *M. ridens* were released at 40 sites in 7 regions in New Zealand (Hawke's Bay, Gisborne, Nelson, Central Otago, Waikato, Wairarapa and Auckland) from 2012-2017 (Table 5.5.1). Parasitoids were released into unsprayed sites of apple trees with large numbers of codling moths. The optimum time to release *M. ridens* adults is January to May, coinciding with maximum numbers of moth larvae.

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Table 5.5.1. Number of *Mastrus ridens* adults released into 7 regions in New Zealand. The numbers of release sites in each region are in parentheses.

Region	Oct. 2012 - Feb. 2013	January-May 2014	January-May 2015	January-May 2016	February-March 2017
Hawke's Bay	8500 (2)	36000 (4)	22500 (10)		
Gisborne	1600 (2)		14000 (7)		
Nelson/Motueka		34000 (6)	5240 (3)		
Central Otago		16000 (2)	28000 (5)		
Waikato			970 (4)		22800 (3)
Wairarapa			1500 (1)		
Auckland				39000 (17*)	
Total number released	10100	86000	72210	39000	22800

Seasonal activity and establishment of *Mastrus ridens* at the release sites: During 2014-15, seasonal activity of *M. ridens* at release sites was determined using 'sentinel bands' (five laboratory-reared diapausing codling moth larvae cocooned in corrugated cardboard strips of 2 cm wide, 30 cm long). Five bands were deployed monthly from June 2014 to June 2015 at each release site. The bands were stapled directly onto trees in the release zones, either around the trunk, or around medium-sized branches. The 'sentinel bands' were protected from bird and mammal predators by a 'collar' of either netting (wire or plastic) or shade cloth wrapped around the band.

Establishment of *M. ridens* was monitored in 2016 by using 'wide bands' (10-cm wide corrugated cardboard strips wrapped around the trunks of apple trees to catch wild codling moth larvae). A protective wire mesh collar was placed around the bands to prevent predation of host larvae. The wide bands were deployed in January 2016 at sites where *M. ridens* had been released between 2012 and 2015. Deployment was timed to catch larvae from the uni-voltine codling moth populations in Hawke's Bay, Gisborne, Nelson and Central Otago regions, and from the second generation of bi-voltine populations in the Waikato region. Fifty percent of the bands at each site were retrieved at the end of summer (early June) 2016. Predation and mortality over winter was determined by retrieving the remaining bands in spring (October 2016) prior to codling moth pupation and emergence. A total of 549 wide bands were retrieved from the five regions.

Retrieved sentinel and wide bands were returned to the laboratory where numbers of surviving and parasitized codling moth larvae were recorded. Retrieved sentinel codling moths were held at 20°C, and the numbers and species of emerging parasitoids recorded.

Mastrus ridens establishment was confirmed in four regions (Table 5.5.2). In those regions, the female parasitoids actively sought and attacked codling moth larvae from at least September to May. This seasonal activity indicates that they may complete several generations a year (compared with only one or two of its host codling moth). Such activity is a feature of effective parasitoid biocontrol agents. Monitoring in Waikato and Auckland is continuing in 2017 to confirm establishment.

Table 5.5.2. Establishment of *Mastrus ridens* in different regions of New Zealand recorded from 'sentinel bands' in 2014, 2015 and from 'wide bands' in 2016.

Region	***M. ridens* recovered from 'sentinel bands'**		***M. ridens* recovered from 'wide bands' in 2016**		
	# *M. ridens* in 2014 (# of sites)	# *M. ridens* in 2015 (# of sites)	# wild cm* recovered (# of sites)	# wild cm* parasitised by *M. ridens* (# of sites)	Average % parasitism
Hawke's Bay	32 (2)	26 (1)	601 (12)	176 (6)	37.9
Gisborne			62 (5)	3 (2)	20.1
Nelson/Motueka	209 (5)	112 (6)	2872 (8)	350 (6)	17.1
Central Otago			1194 (5)	13 (4)	10.4

*codling moth larvae

Existing parasitiod species: The presence of four other species of codling moth parasitoids was reported from sentinel and wide band data. *Liotryphon caudatus* (Ratzeburg) (Hymenoptera: Ichneumonidae), a long-established larval parasitoid from past biocontrol programmes, was commonly found in Hawke's Bay, Gisborne and Waikato. An introduced egg/larval parasitoid, *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae), was found commonly in Nelson and Central Otago, and in low numbers in Hawke's Bay. Low parasitism by the introduced *Glabridorsum stokesii* (Cameron) (Hymenoptera: Ichneumonidae), a pupal parasitoid of many tortricids, was found in Hawke's Bay. *Dibrachys microgastri* (Bouché) (Hymenoptera: Pteromalidae) was recovered from Central Otago, Nelson, and Waikato, as a primary parasitoid from codling moth larvae and as a hyperparasitoid from *M. ridens* cocooned larvae. The very polyphagous *D. microgastri*, a recent accidental arrival would never be considered for introduction. How these parasitoids interact, and how they might affect the impact of *M. ridens*, have not been investigated. The ecological interactions of codling moth and its natural enemies in unmanaged apple blocks will become a focus for future research .

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5.6 Exotic or Native? Interspecific Competition in the Parasitization of the Fruit Fly *Ceratitis cosyra*

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Multiple natural enemies of a pest might enhance the pest control or in contrast be less efficient for the management of the nuisance. Additive reduction of the pest might occur if natural enemies co-inhabitat and do not compete for space or nutrients. Parasitoids obligate dependence on the same host for its survival may possibly generate intra- or interspecific competition when the number of parasítoids per host reduces the availability of food and thus affects the survival, fecundity, growth and development of one or both parasitoids as well as the host species (Aluja *et al.*, 2013). Interspecific competiton is more likely to occur between parasitoids if they develop within the same host, and if they are have similar habitat requirements. Introduction and release of an exotic parasitoid that shares a habitat and host with a native parasitoid might be affected by interspecific competition, which might affect the effectiveness of the biological control agent (Miranada *et al.*, 2015). *Fopius arisanus* Sonan and *Fopius caudatus* Szèpligeti (Hymenoptera: Braconidae) are two solitary endoparasitoids of tephritid fruit flies that parasitize eggs of fruit flies infesting the same fruits. We studied intraspecific competition between these two parasitoids species in the mango fruit fly *Ceratitis cosyra* (Walker) (Diptera: Tephritidae). Based on previous documentation of the equal chance of survival between *F. arisanus* and *Fopius ceratititvorus* Wharton (Hymenoptera: Braconidae) while co-parasitizing (Bokonon-Ganta *et al.*, 2005), we hypothesized that we would not find a superior competitor between the two parasitoids.

Both *F. arisanus* and *F. caudatus* are egg-pupal parasitoids of tepritid fruit flies. The former is a known Asian biological control agent, released on three continents and the latter is a parasitoid of African origin, occasionally tested as a biological control agent. Releases of *F. arisanus* have been made during the last ten years in Africa, i.e., Senegal, Benin, Cameroon, Kenya and Mozambique (Kibira *et al.*, 2015; Ndiaye *et al.*, 2015; Gnanvossou *et al.*, 2016). *Fopius arisanus* was brought to Africa after the invasion of the Asian *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) because this wasp parasitizes *B. dorsalis* both in its native environment and in Hawaii, etc.. *Fopius arisanus* also parasitize other tephritid fruit fly species, such as *Ceratitis capitata* (Wiedemann), *C. cosyra* and *Anastrepha* (Rousse *et al.*, 2006). *Fopius caudatus* parasitizes *C. capitata* and *C. cosyra*

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that infest mango, coffee and other cultivated and wild fruits (Wang *et al.*, 2004; Vayssières *et al.*, 2011).

We designed preference tests and competition experiments in the laboratory with the two parasitoids. In the preference test, we studied their preference for developmental stages of *C. cosyra*, presenting eggs at diffent intervals after fruit infestation. Preference between parasitized and non-parasitized *C. cosyra* infested fruits were also conducted. Their parasitizing ability and survival after co-parasitizing the fruit fly eggs were observed. Dissections of the eggs and first instar larvae (Fig. 5.6.1) revealed the parasitism rate.

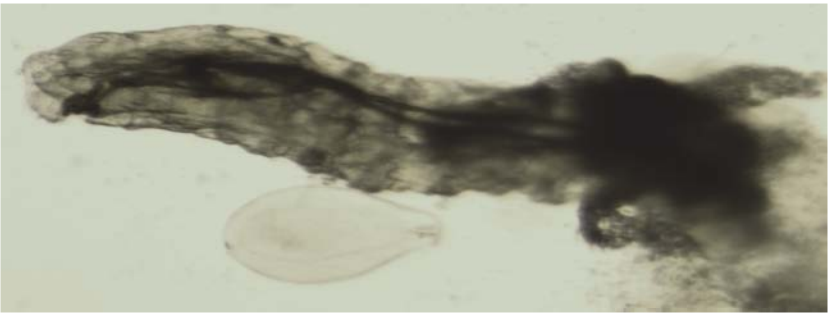

Figure 5.6.1. *Fopius caudatus* egg dissected from 1st instar *Ceratitis cosyra* larvae.

Parasitization by *F. arisanus* and *F. caudatus* resulted in 10-35% mortality of host eggs, depending on species and age of the host egg. Emergence of *F. caudatus* was higher then for *F. arisanus*, i.e., a higher rate of eggs laid by *F. caudatus* developed and emerged as adults. Multiparasitism, i.e., eggs of the two species ovipositied in the same fruit fly egg, of 8% was observed. *Fopius arisanus* avoided super- and multi parasitism more than *F. caudatus* which is an disadvantage for the latter wasp, since only one parasitoid can emerge from one parasitized fruit fly. *Fopius caudatus* oviposition was not lower in hosts previously parasitized by *F. arisanus*, suggesting that *F. caudatus* can not discriminate against parasitized hosts. Behavioural observations showed that *F. arisanus* won the extrinsic competitions against *F. caudatus* as it was more active and chased away its cogener from searching on the oviposition substrate. Our experiments revealed that *F. arisanus* is a stronger competitor than *F. caudatus* in some cases, while survival in *C. cosyra*. is superior for *F. caudatus* than for *F. arisanus*. These results support our hypothesis that the two *Fopius* species have complementary capacities for competiton and we demonstrate the first results of interaction between these two endoparasitoids.

Competitive superiority of early acting parasitoids species in fruit flies (Wang and Messing, 2003) is one of the reasons that the egg parasitoid *F. arisanus* has had a superior establishment record than other braconids that attack various larval instars of fruit flies and why it is widespread for use in biologcal control programs. The strategy to parasitize eggs is rare among fruit fly parasitoids. The knowledge about the range of fruits parasitized by the two parasitoids in Africa is scarse, yet it is known that both wasps parasitize fruit fly eggs in mango, which is infested by both *C. cosyra* and *B. dorsalis*. *Fopius caudatus* parasitism in mango in Benin was almost 10% (Vayssières *et al.*, 2011) while early records of recovery of *F. arisanus* in mango is less than 1% 1-3 years after release (Ndiaye *et al.*, 2015). Their choice of fruit fly host and their survival in them, together with habitat preferences, will further define the occurance of interspecific competition, its outcome and possibilities for co-existence. Our study is the first comparison of the two closely related parasitoid species. Further studies and survillance will show if the competition and niche differences will cause spatial displacement or host shifts as a response to competition avoidance.

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Session 6: Frontiers in Forest Insect Control

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6.1 Investigating the Complex Gall Community of *Leptocybe invasa*

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Leptocybe invasa Fisher & La Salle (Eulophidae: Hymenoptera) is a gall wasp that infests *Eucalyptus* trees. Females oviposit in the midrib, petiole or stem of eucalypt trees, resulting in the development of galls (Mendel *et al.*, 2004). Interestingly, although assumed to be native to Australia due to their association with *Eucalyptus*, the insect was first described outside its native range, when it was reported in Israel in 2000. It subsequently spread to other eucalypt growing areas and is now present in all continents where eucalypts are grown. In these areas *L. invasa* has become one of the most serious pests of eucalypt trees, where high infestations of this insect result in stunted growth, deformation and in severe cases tree mortality (Fig. 6.1.1).

Fig. 6.1.1. Damage to trees from infestation of *Leptocybe invasa*. Trees in foreground are of a susceptible eucalypt clone and those in the background are of a resistant *Eucalyptus* clone.

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Classical biological control has been one of the main management responses in the invasive range of *L. invasa*. Following its introduction in Israel, natural enemies were sought for in Australia, which resulted in the introduction of *Quadrastichus mendelli* Kim & La Salle, *Selitrichodes kryceri* Kim & La Salle (Hymenoptera: Eulophidae) and two *Megastigmus* species (Hymenoptera: Torymidae) (Kim *et al.*, 2008). Subsequent to these introductions, another natural enemy, *Selitrichodes neseri* Kelly & La Salle (Hymenoptera: Eulophidae), was found in Australia and released in South Africa (Kelly *et al.*, 2012; Dittrich-Schröder *et al.*, 2014). These biological control agents have since spread naturally and / or been intentionally released into other countries where *L. invasa* occurs.

Post-release monitoring of *S. neseri* and ad hoc collections in South Africa revealed the presence of other wasps co-inhabiting *L. invasa* galls. These include two *Megastigmus* species: *Megastigmus zebrinus* Grissell, reported to be native to Australia but previously reported in South Africa from seed capsules of *Eucalyptus camaldulensis* Dehnhardt (Myrtaceae) (Klein *et al.*, 2015); and *M. pretorianensis* Doğanlar, recorded as a new species native to South Africa (Dogãnlar, 2015). *Quadristichus mendelli*, released in Israel as a biological control agent, was also reported in South Africa and is assumed to have been accidentally introduced with the pest. In addition, another lineage of *L. invasa* and potentially a different species of *Leptocybe* (Nugnes *et al.*, 2015) was recently detected in South Africa. The distribution and roles of these different wasp species associated with the *L. invasa* galls in South Africa, and specifically the interaction between these species, is currently unknown.

We collected galled material to investigate the distribution and interactions of the various wasps associated with the *L. invasa* complex gall community (Fig. 6.1.2). As it was not feasible to identify the species based on the morphology of the minute larvae, specific primers and restriction enzyme markers were developed to distinguish between species and the two *Leptocybe* lineages. The data reveals that *S. neseri* is well established throughout the country. However, in some sites the galls were dominated by *Megastigmus* species, which are potential parasitoids and / or inquilines within the galls. *Quadrastichus mendeli* has not yet spread to the major eucalypt growing areas, but could potentially contribute to the management of *L. invasa* in South Africa.

Fig. 6.1.2. The complex community associated with *Leptocybe invasa* galls in South Africa, including two distinct lineages of *L. invasa*, possibly representing different species. (Photos of wasp species, S. Bush).

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6.2 Larval Parasitoids for Biocontrol of Invasive Paropsine Defoliatiors

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Many countries have a large *Eucalyptus* plantation forest industry. In New Zealand, *Eucalyptus* form a small component of the plantation estate at present (FOA, 2016), but the genus is becoming increasingly important for providing speciality hardwood products. In a trend similar to other countries (Wingfield *et al.*, 2008) but exacerbated by New Zealand's close geographic proximity to Australia, numerous *Eucalyptus* pests have successfully invaded, including Chrysomelidae (Coleoptera) beetles (Withers, 2001). Of these, five are paropsines on *Eucalyptus*: *Paropsis charybdis* Stål (first found in 1916), *Trachymela sloanei* (Blackburn) (first found in 1976), *Trachymela catenata* (Chapuis) (found in 1992), *Paropsisterna beata* (Newman) (found in 2012) (Yamoah *et al.*, 2016), and *Paropsisterna variicollis* (Chapuis) (found 2016) (Lin *et al.*, 2017). Of all the eucalypt insect pest species, *P. charybdis* has been the most damaging to date, defoliating numerous species of valued Symphyomyrtus eucalypts. The biological control of *P. charybis* has been variable despite egg parasitoids and a ladybird being introduced, both on purpose (*Enoggera nassaui* Girault (Hymenoptera: Pteromalidae) and *Cleobora mellyi* (Mulsant) (Coleoptera: Coccinellidae) and accidentally (*Neopolycystus insectifurax* Girault, Hymenoptera: Pteromalidae) from Australia (Bain and Kay, 1989). Improved control is needed, especially an agent that targets the larval life stage.

Research has since focused on field and laboratory studies of larval parasitoids of paropsines in Tasmania, particularly the solitary larval parasitoid, *Eadya paropsidis* Huddleston and Short (Hymenoptera: Braconidae) (Rice and Allen, 2009). This wasp is univoltine and attacks all larval instars of the first generation of paropsine beetles feeding on *Eucalyptus* in Australia. *Eadya paropsidis* has been reared from a number of paropsine beetles collected from different regions in Tasmania (De Little, 1989; Tanton and Epila, 1984).

Sentinel larval trials and field collections from a number of locations across Tasmania, as well as colour differences in cocoons and adult wasps (G. Allen, unpublished data), indicate the likelihood of multiple species of *Eadya* targeting different paropsines, many of whom are recent invaders elsewhere in the world. A molecular phylogenetic approach with extensive field collections and laboratory rearing is currently being used to explore *Eadya* species and their host relationships (L. Peixoto, B. Sharanowski, unpublished data).

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Pest paropsines in the genera *Paropsis* and *Paropsisterna* are unique to Australia. Phylogenetic relationships of the Chrysomelidae are resolved to the level of family and subfamily, and phylogenetic analyses agree the sister group to Chrysomelinae is Galerucinae (Reid, 2014, 2006). For this reason any country exploring biological control options for paropsine pests will need to become familiar with their endemic and beneficial insect fauna of both subfamilies. In the case of New Zealand, a number of uncommon native beetle species do exist, though phylogenetically they fall in a separate clade to the paropsines (Withers *et al.*, 2015) (Table 6.2.1).

Table 6.2.1. Genera of Chrysomelidae in New Zealand that are most closely related to the tribe Paropsini (target pests) (Withers *et al.*, 2015).

Subfamily	Genus	Status in NZ
Chrysomelinae	*Paropsis, Paropsisterna, Dicranosterna, Trachymela, Peltoschema*	All exotic pests
Chrysomelinae	*Allocharis, Aphilon, Chalcolampra Caccomolpus, Cyrtonogetus*	Endemic, uncommon (uncertain spp.)
Chrysomelinae	*Chrysolina, Gonioctena*	Weed biological control agents (4 spp.)
Galerucinae	*Lochmaea, Agasicles*	Weed biological control agents (3 spp.)
Galerucinae	Multiple	Endemic, root feeders (100 spp.)

New Zealand has also introduced a number of beneficial weed biological control agents in these two subfamilies. These been the focus of host range testing of *E. paropsidis*, especially species with leaf feeding larvae present at the same time of year (early summer) that *E. paropsidis* is active (Withers *et al.*, 2015).

In addition to field research in Tasmania on host-parasitoid relationships, we have undertaken host range testing of *E. paropsidis* within a containment facility in New Zealand against seven non-target species (Withers *et al.*, 2015). Adult females have been subjected to the following assays:

1. no choice physiological assays following 24 hour exposure of 8 larvae to one female parasitoid, rearing larvae to pupation, dissecting all larvae that died prior to pupation for evidence of parasitism. Positive controls consisted of 8 target larvae observed to have each been attacked once by a female parasitoid.
2. sequential no-choice behavioural observations – 10 min observations of 8 larvae to one female parasitoid, alternating order of presentation, either target larvae or non-target larvae first, in a glass arena. When attacks were observed, larvae were reared to pupation, and all larvae that died prior to pupation were dissected for evidence of parasitism. Negative controls consisted of 8 larvae not attacked reared to pupation.
3. two-choice behavioural observations – 25 min observations of 8 larvae each of target and non-target on their host plant in the same glass arena as one female parasitoid, plants not touching. When attacks were observed, larvae were reared to pupation and all larvae that died were dissected for evidence of parasitism.

Preliminary data analysis suggests *E. paropsidis* shows consistent behavioural responses only towards the *Eucalyptus* leaf feeding paropsines. Therefore it shows promise as potentially host specific, the most important aspect for success of New Zealand's biological control project against *P. charybdis*. No "new" biological control agent can be released however until the government agency responsible (Environmental Protection Authority) approves an application containing all the necessary data. This application is planned for 2018. The *Eadya* genus from Tasmania is thought to hold other species that could also be useful biological control agents for other invasive pest paropsines around the world, such as *Paropsisterna selmani* (Reid and De Little, 2013) and *Pst. variicollis*.

Figure 6.2.1. Female *Eadya paropsidis* attacking *Paropsis charybdis* second instar larvae.

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6.3 Biological Control of the *Gonipterus scutellatus* Species Complex: Testing the Species, Climatic or Phenological Mismatch Hypotheses

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Successful biological control of herbivore pests relies on the correct identification of both the host and the parasitoid species (Stiling, 1993; Aebi *et al.*, 2007). In addition, asynchrony between host and parasitoid due to differences in their climatic optima and thermal thresholds may have negative impacts on the success of biological control programs (Godfray *et al.*, 1994; Hance *et al.*, 2007; Thomson *et al.*, 2010). Therefore, understanding the thermal thresholds of both host and parasitoid is important in developing a successful biological control program as well as its sustainability in the light of climate change and continued potential spread.

The Eucalyptus leaf weevil, *Gonipterus scutellatus* Gyllenhal (Coleoptera: Curculionidae), species complex has been economically controlled using the egg parasitoid *Anaphes nitens* Girault (Hymenoptera: Mymaridae), first released in South Africa in 1926 (Tooke, 1953). However, population outbreaks have been observed in the invasive range of *Gonipterus* species over the last couple of decades. The *G. scutellatus* species complex was once treated as a single species, but is now recognised as a cryptic species complex consisting of ten different species native to Australia (EPPO, 2005, 2012; Mapondera *et al.*, 2012). Three of the cryptic species are invasive, *G. platensis* Gyllenhal, *G. pulverulentus* Lea and *Gonipterus* n.sp. 2 with varying distributions (Mapondera *et al.*, 2012). Limited information is available regarding the distribution of the cryptic species and their natural enemies in the native range.

Climate is a potential driver of variable parasitism rates: *Gonipterus* outbreaks can occur at high altitudes in spring, due to a decrease in *Gonipterus* activity and consequently host availablility in winter. Therefore, *Gonipterus* population outbreaks occur in spring before *A. nitens* builds up in numbers (Tooke, 1953). At low altitudes, winter temperatures are mild, and the beetles remain active throughout the year (Tooke, 1953; Tribe, 2005). However, population outbreaks have been observed in summer at low altitudes. The aim of this study was to further investigate the hypothesis that a species mismatch and/or climatic and phenological mismatch drive variable parasitism rates.

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Gonipterus adults and egg capsules were collected from *Eucalyptus* (Myrtaceae) trees in eastern Australia. Parasitoids were reared under controlled conditions and identified. Male adult weevils were identified based on their genitalia morphology and females were identified using DNA barcoding. Upper and Lower thermal threshold were determined for *Gonipterus* species.

Four of the cryptic species and three parasitoid species, including *A. nitens* were collected with varying distributions within the native range. Differences in thermal thresholds were observed between the different *Gonipterus* species. Thermal constraints, and thus, temperature plays an important role in the distribution of insects. Differences in thermal limits between pest and parasitoid may have implications in phenological asynchrony in biological control (Thomas and Blanford, 2003). In addition, exposure to extreme temperatures may have a negative impact on parasitoids by decreasing longevity, fecundity, mobility and increase production of males (Hance *et al.*, 2007). This may in turn lead to a reduced suppression of the host population and lead to population outbreaks. In conclusion, results of the current study will provide insights in the selection and development of additional biological control agents better suited for variable climatic conditions and more robust to climate to change.

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6.4 A Successful Case of Classical Biological Control of a Gall Wasp

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The Asian Chestnut Gall Wasp (ACGW), *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), represents a noticeable exception to the rule that gallmakers are of relatively little economic importance as, in the invaded countries, it poses a serious threat to all *Castanea* (chestnut) species and their hybrids. Chestnut is a global nut commodity, used for thousands of years by humans as a staple food and it is still fundamental as typical element of local cuisine. Chestnut species are also cultivated for both timber and honey production as well as for ornamental purposes. They are also essential components in animal food webs and in forest ecosystem communities. In particular, European chestnut, *Castanea sativa* Miller (Fagaceae), was often associated with early farming settlements in southern Europe (Battisti *et al*., 2014). As heritage of this ancient traditional land use and management, nowadays Europe includes four of the top 10 chestnut-producing countries worldwide, with Italy, Greece, Portugal, and Spain accounting for about 6 % of global nut production after China, South Korea, Turkey, and Bolivia (Bento *et al*., 2016).

After colonizing Japan, Korea, North America, and Nepal (in the 1940s, 1950s, 1970s, and 1990s, respectively), *D. kuriphilus* arrived in 2002 in northwestern Italy, from where it spread throughout the country and Europe. In Veneto (northeastern Italy), the ACGW was first reported in 2007. Eradication attempts failed and in a few years the pest expanded its distribution, the new range perfectly overlapping that of the European chestnut, an area of about 20,500 ha, with high infestation levels in all chestnut stand types. Surveys were conducted to assess the economic impact of the invader, as chestnut growers complained that nut yield had greatly decreased, and a positive linear relationship was found between attack level and yield loss, the latter approaching 80 % when more than 6 galls per 50-cm twig were found (Battisti *et al*., 2014). Furthermore, a decline in basal area increment (BAI) as high as 40 % was assessed in coppice stands (Battoni, 2016; Marcolin *et al*., 2017) and, again, a noticeable reduction in honey yield was recorded although not supported by quantitative data.

As the regulation exerted by the native natural enemies was very low, ranging from 0.11 % to 1.86 % (mean 0.45 ± 0.35 %) 3–5 years after colonization (Colombari and Battisti, 2016a), a classical biological program was implemented in 2010 using the non-native parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) (Gibbs *et al*., 2011). Almost 500 introductions, each consisting of 100 females and 50 males, were made in six years by releasing individuals only once at each site on 1–2 infested chestnut trees (Fig. 6.4.1). The propagule size was good enough to have the parasitoid successfully established at all release sites where it persisted, reproduced and enlarged its range by active flight (up to 650 m) over the next few years following the release. Moreover, monitoring carried out

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at non-release sites demonstrated that the parasitoid could colonize new areas over long distance aided by winds ('jump dispersal'), as it was collected up to 70 km away from the nearest release site 1-2 years after the introduction (Colombari and Battisti, 2016b). Despite initially low numbers, probably because of interactions with some native parasitoids that may act as facultative hyperparasitoids and to migration and area-dilution effect, *T. sinensis* abundance then sharply rose at all sites. The infestation rates in the surveyed stands showed an average decrease of 50 % between 2014 and 2015, when percentage of parasitism of larval chambers ranged from 60.7 % to 90.8 % (mean 82.4 ± 1.72 %) without significant differences among sites in relation to host density, the year of *D. kuriphilus* infestation or the year of *T. sinensis* release or arrival. Remarkably, there was also an increase in the average percentage of parasitism by native species (from 0.45 % to 2 %) that contributed to pest mortality up to 7 %.

Although predicted to take almost eight years to show impact (Moriya *et al.*, 1989), the pest was thus controlled within 3 to 5 years after the establishment of *T. sinensis*, resulting in recovering about 50 % of yield loss. Reasons for the success of the parasitoid include: mainly host specificity, dispersal ability, and life cycle. *Torymus sinensis* is well-synchronized with the host phenology, having only one generation per year as the ACGW. Conversely, the native parasitoids associated with cynipid gall wasps, especially on oaks, generally perform two generations and do not match well with the single occurrence of galls on the chestnut. Moreover, its great rate of spread, as a result of a combination of short- and long-distance dispersal mechanisms (stratified dispersal), can ensure a rapid colonization of the host geographic range mostly where landscape composition helps the host-finding process (i.e., uninterrupted small and clustered patches of host trees; Colombari and Battisti, 2016a). Another important trait is the possibility to remain in prolonged diapause for more than one year in the old withered galls, facilitating the tracking of fluctuations in time and space of the host population (Ferracini *et al.*, 2017). Among potential negative impacts, it has been claimed that the high density of the parasitoid could negatively affect native gall wasps (e.g., several species associated with oaks). A large survey conducted during a 3-year period has shown that such an impact is so far negligible (Ferracini *et al.*, 2017). All these factors make *T. sinensis* an optimal agent for the biological control of the ACGW in Italy and in Europe, as previously shown in other countries where the pest was unintentionally introduced (Gibbs *et al.*, 2011). For this reason, it has been selected for the biological control of the gallmaker also in Turkey, the third world's producer of chestnuts (Bento *et al.*, 2016), where the ACGW has been first recorded in 2014 (Çetin *et al.*, 2014).

Fig. 6.4.1. *Torymus sinensis* females on galls of *Dryocosmus kuriphilus* soon after the release on a heavy infested chestnut tree.

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6.5 Biological Control of *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae) in *Eucalyptus* Plantations in Brazil: An Update

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South America has suffered problems with many invasive pests in forest plantations, mainly in *Eucalyptus* (Myrtaceae). The bronze bug, *Thaumastocoris peregrinus* Carpintero and Dellapé (Hemiptera: Thaumastocoridae), was detected in Brazil in 2008 (Wilcken *et al.*, 2015) and infested 245,000 ha of eucalyptus plantations in 2012, causing reduction of 10-15 % in wood production and losses of US$ 330 million during 2010-2015. The main management strategy is based in biological control, using the egg parasitoid, *Cleruchoides noackae* Lin and Huber (Hymenoptera: Mymaridae), introduced from Australia in 2012, and native predators and entomopathogenic fungi (Wilcken *et al.*, 2015).

The egg parasitoid *C. noackae* is the main biocontrol agent and it has been reared in the laboratory and released throughout the country. This parasitoid has arrenotokous parthenogenesis and an adult longevity of 1.1 and 3.6 days without and with food, respectively (Mutitu *et al.*, 2013; Souza *et al.*, 2016). The life cycle duration of *C. noackae* (egg-adult) varies according temperature (L.K. Becchi, 2017, pers. comm.). Bioassays and field evaluations showed a parasitism rate of 50-60% by *C. noackae* (Barbosa *et al.*, 2017) (Table 6.5.1).

Table 6.5.1. Total number of eggs and adults, adults emergence (%), and sex ratio (mean ± SE) of *Cleruchoides noackae* from *Thaumastocoris peregrinus* eggs per day (Barbosa *et al.*, 2017).

Site	Eggs (n)	Adults (n)	Emergence (n)	Sex ratio
Laboratory	230	123	53 ± 3	0.69 ± 0.02
Field	660	342	52 ± 3	0.65 ± 0.02

The egg parasitoid was released in ten Brazilian states, releasing 33,438 individuals in 2014, 118,432 individuals in 2015, 100,400 individuals in 2016 and 4,000 individuals until May, 2017, totaling 256,270 parasitoids released.

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Figure 6.5.1. *Cleruchoides noackae* female parasitizing egg of *Thaumastocoris peregrinus*.

Native predators were recorded preying on nymphs and adults of *T. peregrinus* in both field and laboratory conditions. Larvae of *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) and nymphs of *Supputius cincticeps* (Stål) (Heteroptera: Pentatomidae) preyed on 10.4 and 10.3 *T. peregrinus* nymphs, respectively, during 24 hours (Barbosa *et al.*, 2010; Souza *et al.*, 2012), and *Atopozelus opsimus* Elkins (Hemiptera: Reduviidae) preyed on two adults of *T. peregrinus* during one hour (Dias *et al.*, 2014).

Considering microbial control, entomopathogenic fungi, such as *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Clavicipitaceae), have been used in aerial spraying and natural epizootics of *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, *F. equiseti* (Corda) Saccardo (Nectriaceae) (Velozo, 2015) and *Zoophtora radicans* (Brefeld) Batko (Entomophthoraceae) (Mascarin *et al.*, 2012) have caused extensive mortality of the pest.

After four years of initial releases of *C. noackae* in eucalyptus plantations in Brazil, the infested area has been reduced to 18.8 % (app. 46,000 ha) compared to 2012 data, demonstrating the effectiveness of classical biological control of bronze bug in Brazil.

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6.6 Ecology and Biological Control of Outbreak Populations of Winter Moth in the Northeastern United States

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The winter moth, *Operophtera brumata* L. (Geometridae: Lepidoptera), a leaf-feeding inchworm native to Europe, invaded the northeastern USA about 20 years ago and has caused widespread defoliation of many deciduous trees ever since (Elkinton *et al.*, 2010, 2015). The winter moth has continued to spread west and south across Massachusetts and Rhode Island (Elkinton *et al.*, 2014). In 2012, outbreaks occurred for the first time in southeast Connecticut and coastal Maine (Elkinton *et al.*, 2015).

Beginning in 2004, we have collected yearly life table data on winter moth from red oak, *Quercus rubra* L. (Fagaceae) and red maple, *Acer rubrum* L. (Sapindaceae), two of the dominant deciduous trees in our forests, at 12 sites that have been heavily infested in eastern Massachusetts. Analyses of those data show that winter moths fluctuate with a biannual cycle of high and low densities. Mortality is strongly density dependent in the larval stage, but not consistently density dependent in the pupal stage. Overwintering mortality is also density dependent. Research by Pepi *et al.* (2016) showed that mortality in the larval stage and the overwintering stage were both dominated by density-dependent larval dispersal. Other forms of larval mortality, such as by pathogens or predators, were insignificant. Overcompensating density dependence in this stage explains the biannual cycle of density evident in our data and in previously published data from Nova Scotia (Embree, 1965).

Prior to the current winter moth invasion in the northeastern United States, there had been three previous invasions to North America: Nova Scotia in the 1930s, Oregon in the 1950s and to British Columbia in the 1970s. All three prior invasions have been suppressed by the introduction of parasitoids from Europe, in particular the tachinid fly *Cyzenis albicans* (Fallén) (Diptera: Tachinidae), and low-density populations of winter moth now persist indefinitely in these regions, similar to those in most of Europe (Roland and Embree, 1995). We have introduced many thousand *C. albicans* distributed across 43 sites in eastern Massachusetts, Rhode Island, Connecticut and Maine (Fig. 6.6.1), and it has established at 21 of those sites. As reported from Nova Scotia in the 1950s (Embree, 1965), it typically takes 3 to 5 years before recovery of *C. albicans* at release sites. Since there is only one generation per year of both the fly and the winter moth, it takes several years for the 1500-2000 flies released at a site to catch up with the millions of winter moths that exist at that site. We have now recovered the fly at all 17 of the sites where it was released prior

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to 2012 (Fig. 6.6.1). At several of those sites, establishment of *C. albicans* was documented for the first time in 2016. We expect it will soon be established at all, or most, of the 43 release sites.

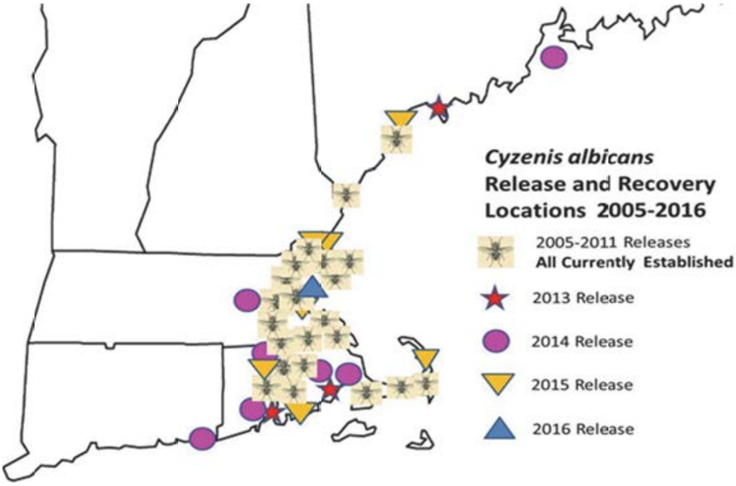

Fig. 6.6.1. *Cyzenis albicans* release and recovery locations in the northeastern USA.

Prior to 2015, *C. albicans* for release in the northeastern United States were obtained from Vancouver Island, British Columbia, where it had been established in the 1970s. Starting in 2015, however, we switched our collection efforts to Wellesley, Massachusetts, where high levels of parasitism were documented, comparable to what we encountered in British Columbia. Furthermore, the flies we collect in Wellesley are presumably better adapted to northeastern climatic conditions. Late-instar winter moth larvae are collected at sites where parasitism by *C. albicans* is high and the larvae are reared to the pupal stage. *Cyzenis albicans* pupates inside the winter moth pupae. The fly puparia are kept over the winter and adult flies released the following spring.

Each year 100-500 late-instar winter moth larvae are collected from each of the now 43 previous release sites. Larvae are reared to the pupal stage and dissected in mid-summer to document establishment of *C. albicans* and to measure percent parasitism. Parasitism at a release site in Wellesley, Massachusetts has fluctuated between 15 and 40% over the past 5 years. In 2017, both defoliation and the densities of winter moth pupae remained low at that site. Levels of parasitism at several of our older release sites had increased markedly in 2016 compared to previous years and now exceed 25%, which is the level at which effects on winter moth density in Wellesley were observed over the past five years. Furthermore, winter moth densities across many sites declined dramatically in 2016-2017. These data suggest we are on the verge of converting winter moth into a non-pest in the areas where *C. albicans* is established

Roland (1988, 1990) and Roland and Embree (1995) published an intriguing analysis of the successful biological control of winter moth in Canada by *C. albicans*. Roland claimed that the reduction in winter moth density was caused mainly by pupal mortality due to predators and not the direct effect of *C. albicans* parasitism. This pattern was true both in Nova Scotia and on Vancouver Island. He proposed that the impact of *C. albicans* parasitism was enhanced by the action of soil predators in the form of predatory beetles. For example, he hypothesized that these generalist predators are able to regulate winter moth densities only when *C. albicans* has reduced those densities to a manageable level. Our preliminary data from the release site in Wellesley supports this idea. Winter moth densities at this site have declined by 95% over the past five years, whereas parasitism by *C. albicans* has varied between 15% and 48 % over the same time interval. That means that parasitism alone cannot account for this large drop in density.

Predation experiments (Broadley, unpublished data) involving deployment of winter moth pupae at this and other sites will determine if Roland's ideas are correct. High levels of predation by invertebrate and vertebrate predators have been documented at these sites. This work is described on this system in a follow-up talk (This volume, 7.1) at this symposium. The research of Broadley and Roland illustrates why it is important to understand the impact of *C. albicans* in the context of all the other causes of mortality occurring in the winter moth system. What the prognosis is for winter moth densities in the northeastern United States remains to be seen, but it is vital that we try to quantify and explain the other factors influencing winter moth densities, in addition to *C. albicans*. We are hopeful that the introduction of *C. albicans*, in conjunction with these other native sources of mortality, will convert winter moth into a non-pest, much as it apparently did in the three previous introductions to North America.

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Session 7: Biocontrol Marketplace I – Free Topics

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7.1 Friend or Foe: The Role of Native, Natural Enemies in the Biological Control of Winter Moth

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Natural enemies that cross over from related native species to invasive species mediate invasions in complex ways (Strauss *et al.*, 2012; Dearborn *et al.*, 2016; Faillace *et al.*, 2017). They have the potential to slow down invasions and aid in biological control efforts (Kenis *et al.*, 2008; Vindstad *et al.*, 2013; Dearborn *et al.,* 2016). In the northeastern United States, the European winter moth, *Operophtera brumata* L. (Lepidoptera: Geometridae) is an invasive, forest pest causing widespread defoliation in rural and urban settings (Elkinton *et al.*, 2014). Following successful biological control of winter moth in Nova Scotia and British Columbia using the tachinid fly, *Cyzenis albicans* (Fallén) (Diptera: Tachinidae) (Embree 1966; Murdoch *et al.*, 1985; Roland and Embree, 1995), similar efforts are underway in the northeastern United States (Elkinton *et al.*, 2015).

While biocontrol shows promising results, success likely depends on additional mortality from native natural enemies. In the northeast U.S., the closest relative to winter moth is Bruce spanworm, *O. bruceata* (Hulst) (Lepidoptera: Geometridae). The two are congeners and can hybridize (Gwiazdowski *et al.*, 2013; Havill *et al.*, 2017), thus it is likely that natural enemies from Bruce spanworm as well as other generalists could cross over to cause mortality to the winter moth population. However, little is known about the identity or impact of these native species in this system or other systems.

To learn more about the role of native pathogens, parasitoids, and predators, we employed a suite of field and laboratory studies. Over the span of four years, we collected caterpillars of winter moth and Bruce spanworm. From these collections, we quantified mortality in rearing and determined the source of mortality. We then used a subsample of the surviving cohort, to deploy as pupae in the field to determine rates of predation and parasitism in pupae. Remaining live samples were used for lab trials of pathogen and parasitoid host range.

Our results suggest that in its introduced range, winter moth is not affected by pathogens from native congener species (Broadley *et al.*, 2017; K. Donahue and H.J. Broadley, 2017, unpublished results). While Bruce spanworm experienced mortality rates three or more times higher than that of winter moth from virus and microsporidia, we found no evidence that winter moth shares viral or microsporidian pathogens with Bruce spanworm. The rates of mortality on Bruce spanworm were particularly high when Bruce spanworm was at high densities. While pathogens do not seem to be shared between the

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two species, we found that generalist predators and a native parasitic wasp, *Pimpla* sp. (Hymenoptera: Ichneumonidae) cause high levels of mortality, both of which have increased significantly over the four years of the study (H.J. Broadley, 2017, unpublished results). In particular, we expect predation to be density dependent and to play a larger role when winter moth has been reduced to low density populations by the biocontrol agent, *C. albicans* (Roland and Embree, 1995; H.J. Broadley, 2017, unpublished results). We suggest that the recruitment of native predators and parasitoids, in concert with the biocontrol agent, may manage winter moth populations. At the same time, we note that native natural enemies can hinder our biological control efforts. While we have found native parasitoids to overall, have a beneficial effect, we have found that native hyperparasites may hinder biocontrol efforts with *C. albicans* (E. Kelly and H.J. Broadley, 2017, unpublished results). By evaluating the mortality rate and source of mortality on pupae parasitized by *C. albicans*, we found that mortality of *C. albicans* was surprisingly high and primarily due to predation, but also due to hyperparasitism from the generalist Ichneumonid wasps *Phygadeuon* sp., *Pimpla* sp. and *Gelis* sp. (Hymenoptera: Ichneumonidae).

Overall, this research improves our understanding of the relationship between introduced and native natural enemies and their relative contribution to successful biocontrol.

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7.2 BiCEP: Progress in a Global Collaboration for the Biological Control of Australian-origin Eucalypt Pests

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The Biological Control of Eucalypt Pests Alliance (BiCEP) was formed in 2013 to better coordinate the research needs internationally for biological control of eucalypt insect pests that had been moving globally over the previous decade and for other pests that were re-emerging as significant issues. BiCEP is a collaborative alliance of industry and researchers, initially with partners at the Forest Science Research Institute of Brazil (IPEF), Forestry South Africa (through the University of Pretoria), the University of the Sunshine Coast, and the Queensland Department of Agriculture and Fisheries.

Initially we identified the key pests ('the big five') that required further research in Australia and globally. These were: *Leptocybe* gall wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae), bronze bug, *Thaumastocoris peregrinus* Carpintero & Dellapé (Hemiptera: Thaumastocoridae), red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae), Eucalyptus gall wasp, *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae) and the Eucalyptus weevil, *Gonipterus* spp. complex. These were then allocated into those pests that needed basic discovery research (discover new biocontrol agents - e.g., for *T. peregrinus*, *L. invasa*), those that needed application research (better distribution of existing natural enemies - e.g., *L. invasa*, and *O. maskelli*) and those that required fine-tuning research (better-matching of existing natural enemy species and bioclimatic types - e.g., *Gonipterus* species complex and *G. brimblecombei*). Initially BiCEP considered *T. peregrinus* and *L. invasa* to be the highest priority pests, given their more recent introductions in Brazil and South Africa. More recently, *Gonipterus* spp. and *G. brimblecombei* have become of higher importance for BiCEP partners, as the initial efforts to discover new biocontrol agents for *T. peregrinus* and *L. invasa* have borne fruit with the releases of *Cleruchoides noackae* Lin and Huber (Hymenoptera: Mymaridae) (an egg parasitoid of *T. peregrinus*) and *Selitrichodes neseri* Kelly & La Salle (Hymenoptera: Eulophidae) (a parasitoid of *L. invasa*) in both Brazil and South Africa. Partner countries are now monitoring the impacts of these biocontrol agents. As time goes on, biocontrol of these two pests may also require fine-tuning.

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Research Highlights

Bronze Bug *Thaumastocoris peregrinus*: Genetic characterisation of populations of *T. peregrinus* in Australia are now complete, using CO1 and microsatellite analyses, and this work has identified regional populations of this pest that are the likely origin of the Sydney and overseas populations. These regions will now be surveyed to attempt to identify new biocontrol agents and to sample more genetically diverse populations of *C. noackae*.

***Leptocybe* gall wasp:** Surveys have been undertaken of populations of gall wasps and their associates in eastern Australia (from Cairns to Coffs Harbour). Results from these surveys have played an essential role in understanding the global invasion pattern of these wasps. We have yet to find *L. invasa* in Australia, but we have shown that *Leptocybe* sp. 9 (now also spreading globally) is widely distributed in eastern Australia. The impact of the second *Leptocybe* species is as yet unknown. Genetic studies are now completed on the *Quadrastichus* spp. and *Selitrichodes* spp. (Hymenoptera: Eulophidae) parasitoids that are associated with *Leptocybe* sp. 9 in eastern Australia and introduced overseas as biocontrol agents. This knowledge will assist in future selection of parasitoids from these genera should more species, greater genetic diversity or better climate matched biotypes be required in the future. Further genetic studies on another important parasitoid genus of *Megastigmus* spp. (Hymenoptera: Torymidae) collected in Australia surveys and from overseas are now underway and which will provide similar information to aid in future biocontrol of *Leptocybe* spp.

Red gum lerp psyllid *Glycaspis brimblecombei*: This pest is mostly under good control by the parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae), but it does nor persist well under persistent hot, dry conditions (da Silva *et al.*, 2013; Filho *et al.*, 2015; Wilcken *et al.*, 2013). It is therefore thought that better climate matched biotypes of *P. bliteus* from Australia may improve biocontrol of *G. brimblecombei* in these regions. Climate matching of areas in Brazil that have consistently occurring outbreaks of *G. brimblecombei* associated with hot, dry conditions with Australia and South Africa has now been carried out and has identified candidate regions in Australia to survey for *P. bliteus*. Surveys in these areas will commence soon.

Eucalyptus weevil *Gonipterus* species complex: *Gonipterus scutellatus* Gyllenhal (Coleoptera: Curculionidae) was one of the first Australian-origin pests to be introduced to eucalypt growing regions overseas, and had been mostly under good biocontrol by the egg parasitoid *Anaphes nitens* Girault (Hymenoptera: Mymaridae). It has recently been shown that *G. scutellatus* is a complex (Mapondera *et al.*, 2012) consisting of at least ten cryptic species. In recent years, a resurgence of this pest has occurred in many eucalypt growing regions of the world, including South Africa, Brazil, Portugal, Spain, Chile and in Western Australia. Host species and climatic mismatches of the parasitoid may be potentially responsible for this pest resurgence, and new work is under way in Australia and with BiCEP's overseas partners to research these issues and tailor biocontrol strategies to suit.

The first three-year contract of BiCEP has now concluded and partners are currently negotiating a new four-year contract to continue this work. With the rising rate of spread of eucalypt pests around the globe (Hurley *et al.*, 2016) the need for new or better host- or climate-matched biocontrol agents will continue into the future, necessitating long-term collaborations of this type.

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7.3 Introduction of *Tachardiaephagus somervilli*, an Encyrtid Parasitoid, for the Indirect Biological Control of an Invasive Ant on Christmas Island

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On Christmas Island, the invasive yellow crazy ant, *Anoplolepis gracilipes* Smith (Hymenoptera: Formicidae) threatens rainforest biodiversity and ecosystem function through direct predation of the endemic red land crab *Gecarcoidea natalis* Pocock (Decapoda: Gecarcinidae), a keystone species (O'Dowd *et al*., 2003). Multi-queen supercolonies of *A. gracilipes* occupied 25% of the rainforest on the island (Abbott and Green, 2007) with a density of more than 2000 ants per m^{-2} sustained on honeydew from the honeydew-producing scale insects in the canopy (Abbott, 2005). Management of yellow crazy ant supercolonies through chemical control have had mixed success and resurgence of supercolonies in areas treated with toxic bait raised concerns about the effectiveness of chemical control over a sustainable, long-term period (Green and O'Dowd, 2009). Given the mutualism between *A. gracilipes* and the introduced yellow lac scale *Tachardina aurantiaca* (Cockerell) (Hemiptera: Kerriidae) (Fig. 7.3.1.a), the removal of one species should lead to the decline of its mutualistic partner. Suppression of this ant and its impacts may be achieved indirectly through biocontrol of its primary honeydew source, *T. aurantiaca*. Here we describe the rearing and export of a host-specific parasitoid, *Tachardiaephagus somervilli* (Howard) (Hymenoptera: Encyrtidae), from its native range in Malaysia to Christmas Island. This is the first example of introduction and direct release of a biological control agent under Australian jurisdiction that did not involve the use of a quarantine facility in Australia.

Rearing of *Tachardiaephagus somervilli* for export: Loading of crawlers of *Tachardina* on *Acacia* (Fabaceae) plants was done in a rearing facility, where the plants were maintained under grow lights at 27-32°C, 60-80% relative humidity and a photoperiod of L12:D12. There were altogether 29 plants (shoots) with susceptible females of *Tachardina* of various age groups. They were exposed to *T. somervilli* in two batches for the production of the founder population for export. The first batch of F0 adults of *T. somervilli* comprised a total of 38 wild-caught females and were released onto 19 shoots that were enclosed within mesh bags, with two female wasps per bag. The second batch comprised 60 laboratory-emerged *T. somervilli* (29 males and 31 females) that were released onto another 10 shoots enclosed in mesh bags, with a range of 1-5 pairs of wasps per bag depending on

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the number of female scales. Approximately 20 days after the scales were exposed to the wasps, females of *Tachardina* were excised and placed in individual vials to await the emergence of F1 adults of *T. somervilli*. This step was repeated until all female *Tachardina* were excised from the 29 shoots. Each of the emergence vials was examined daily for newly emerged wasps (Fig. 7.3.1.b). Operational protocols were followed to maintain work practices, and the rearing facility and the emergence room were under hygienic conditions to avoid cross-contamination and ensure the safe introduction of the biocontrol agent.

The initial batch of *T. somervilli* for export comprised a total of 177 F1 adults (66 males and 111 females). The wasps were kept in individual vials fitted with a small cotton ball soaked in 30% honey solution. The vials were slotted into Perspex racks fitted into transparent plastic boxes and then inserted into a foam insulated Pelican case (Fig. 7.3.2.a). A second export of F1 adults of *T. somervilli*, a total of 180 F1 adults (42 males and 138 females), was delivered via air freight. Prior to shipment, screening of the wasps was done by Chris Norwood (Australian Department of Agriculture).

Fig. 7.3.1. a) The mutualistic relationship between the yellow crazy ant and the yellow lac scale. **b)** Female *Tachardiaephagus somervilli* (40X magnification, 2 mm in length).

Fig. 7.3.2. a) Wasps were transferred individually into plastic, crush-proof export vials fitted into transparent plastic boxes and then into a Pelican case. **b)** Production facility for rearing of *T. somervilli* on Christmas Island.

Field release of *Tachardiaephagus* and monitoring of control and release sites on Christmas Island: When the wasps arrived on Christmas Island, they were transferred to plants with *Tachardina* in mesh bags in the greenhouse (Fig. 7.3.2.b). Following introduction, the F1 wasps produced a total of 1257 F2 adults (518 females and 739 males) and were reared to the F3+ generation for field releases of about 1000 females at each of four rainforest sites. The total number of female scales excised from plants, which were exposed to F3+ generations of wasps, was divided into two where half of the scales were retained in the laboratory for the production of subsequent generations of wasps while the other half was used for field releases. For field releases, the excised scales were placed into an emergence jar and hung on nylon lines suspended from tree canopies. At the time of writing, it was estimated that more than 10 000 adult wasps have been released at the four rainforest sites. Releases were done at one of each of the paired sites while the other remains as a control.

The outcome of the project, which involves field establishment and level of parasitism by *T. somervilli*, abundance of *Tachardina*, and activity of *A. gracilipes* on the forest floor and on tree trunks, will be monitored at four paired sites in the rainforest, each 5-10 ha in area. Assessment of ant activity on the forest floor and tree trunks is done monthly before and then after release of *T. somervilli*. Ant activity on the ground is assessed through card counts of the number of ants crossing a 10 cm x 10 cm card over a 30-second period while tree traffic is assessed by video-recording ants (using a GoPro camera), for a 30-second period, crossing a marker around the entire trunk circumference. Abundance of *Tachardina* and parasitism levels by *T. somervilli* (estimated by exit holes in *Tachardina*) are assessed on randomly-selected small tree branches shot down from the canopy using a shotgun. This will be done six months after initial release of *T. somervilli* at a site or coincident with changes in ant abundance.

The introduction of *T. somervilli* as a biocontrol agent of the yellow lac scale is seen as a viable, long-term solution to the yellow crazy ant problem on Christmas Island. The effect from the released wasps, however, would not be immediate, therefore a continuous post-release monitoring programme will be important to determine its effectiveness. This project is an example of the international collaboration of multiple agencies in solving cross-border issues.

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7.4 *Orius laevigatus* Induces Plant Defenses in Sweet Pepper

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Pest management in protected sweet pepper crops primarily relies on biological control strategies. The release of the phytoseiid *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) and the anthocorid *Orius laevigatus* Fieber (Hemiptera: Anthocoridae) provides effective control of the two key pests of this crop, the thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) and the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Sanchez *et al.*, 2000; Calvo *et al.*, 2009; van der Blom *et al.*, 2009). A part of their direct effect on pest predation, zoophytophagous predators may induce defensive plant responses due to their plant feeding behaviour which involves the release of diverse volatiles through different pathways that are triggered by phytohormones (De Puysseleyr *et al.*, 2011; Naselli *et al.*, 2016; Pappas *et al.*, 2015, 2016; Pérez-Hedo *et al.*, 2015a,b). These responses may result in the repellence or attraction of pests and natural enemies. It is hence hypothesized that *O. laevigatus* would be able to induce plant responses in sweet pepper as has been demonstrated in other plant-zoophytophage systems. As a first step to better understand the interaction between *O. laevigatus* and sweet pepper, the behavior of *O. laevigatus* on the plants was studied and plant feeding behaviour quantified to compare general behaviors. *Orius laevigatus* spends the majority of its time (38%) feeding on apical meristems and apical fresh leaves, which were also preferred residence locations (Bouagga *et al.*, 2017) (Fig. 7.4.1).

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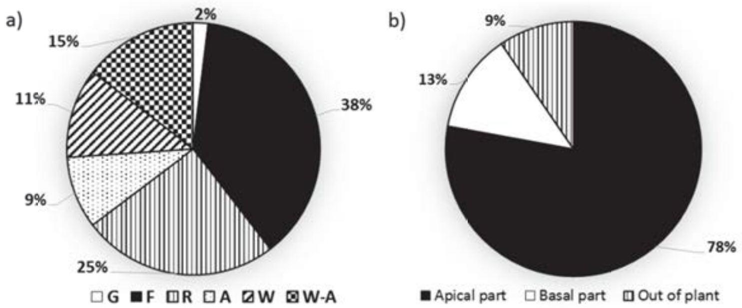

Fig. 7.4.1. a) Percentage of time spent exhibiting different behaviors (G: grooming; F: feeding; R: resting; A: antennating, W: walking; W-A: walking-antennating) by *Orius laevigatus* adults on sweet pepper plants; **b)** percentage of residence time of *O. laevigatus* adults on sweet pepper plants (adapted from Bouagga *et al.*, 2017).

A series of experiments were conducted to determine whether *O. laevigatus* feeding punctures on sweet pepper induce plant defence responses and whether these in turn lead to behavioural responses in pest and natural enemy species. Specifically, *O. laevigatus*-punctured sweet pepper plants induce repellency of the whitefly *B. tabaci* and the thrips *F. occidentalis*. In contrast, the whitefly parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinindae) was significantly attracted to *O. laevigatus*-punctured plants (Bouagga *et al.*, 2017). Our results also showed that the whitefly *B. tabaci* reduced its ovipositing and, more interestingly, its progeny when forced to oviposit on *O. laevigatus*-punctured plants (Fig. 7.4.2). This is a very interesting result that could explain the great success achieved by the IPM programs based on the release, establishment and conservation of *O. laevigatus* in sweet pepper crops.

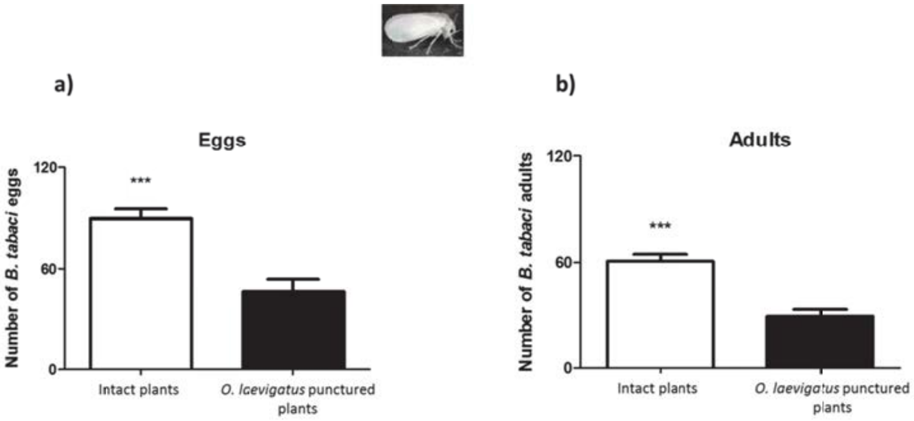

Fig. 7.4.2. Persistence of plant-mediated effect by *Orius laevigatus* on the performance of *Bemisia. tabaci* on *O. laevigatus*-punctured plants. **a)** Number of *B. tabaci* eggs per plant, **b)** Number of B. tabaci adults surviving from the hatched eggs, (mean number ± SE, n=5). Seven couples of B. tabaci were released per cage and left to lay eggs on the plants for 48 hours. (t-test; $P < 0.05$).

In parallel, targeted gene-expression analysis was used on plants previously exposed to *O. laevigatus* to ascertain which signaling pathways could be involved in plant defensive responses. Activation of the jasmonate acid and salicylic acid signalling pathways occurred in those sweet pepper plants previously punctured by *O. laevigatus* (Bouagga *et al.*, 2017).

Finally, the volatile compounds released as part of the plant response to *O. laevigatus* feeding punctures have been also characterized. Untargeted analysis of the volatiles emitted allowed the identification of ten compounds with significantly increased levels in punctured plants, while no compounds with decreased levels were identified. The emission of discriminant compounds increased in the range of 2 to 100-fold and corresponded to terpenoids (1 monoterpenoid, 4 sesquiterpenoids and 1 norisoprenoid), a set of two (Z)-3-hexenyl esters, methyl salicylate and another unknown compound (Bouagga *et al.*, 2017).

In summary, Bouagga *et al.* (2017) described, for the first time, the defensive response that *O. laevigatus* induces in sweet pepper plants due to its phytophagous behavior. This is of special interest since the predator *O. laevigatus* has been one of the most studied and successfully used augmentative biological control agents in sweet pepper. This indirect defense mechanism could add value to the current and future role played by this predator in sweet pepper crops. The practical application of predator-induced plant volatiles for insect pest control remains a goal that will require coordinated research by agricultural scientists and chemical ecologists.

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7.5 The Role of Tomato Plant Volatiles Mediated by Zoophytophagous Mirid Bugs

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Over the past decade, various strategies for biological control of major pests in protected crops in southern Europe have been successfully implemented. Perhaps the two most relevant and recent successes of biological control have occurred in greenhouse tomatoes and peppers in southeastern Spain (Calvo *et al.*, 2012; van der Blom *et al.*, 2009). These successes have occurred primarily through the selection and implementation of native generalist predators from the Mediterranean basin that naturally colonized crops in this area and, therefore, are adapted to local environmental conditions (Pérez-Hedo and Urbaneja, 2015).

The zoophytophagous predators (Miridae) are a special case of generalist predators that can also feed on the plants where they live. This group of predators may use different food resources, being able to feed on more than one trophic level, such as alternative prey and/or plant material. This characteristic facilitates its establishment before a pest infestation and its maintenance in the crops during periods of prey scarcity, resulting in crop systems that are more resilient to pest attacks (Pérez-Hedo *et al.*, 2017).

By feeding on plants, mirid predators, as strict herbivores, can also activate plant defense mechanisms (Naselli *et al.*, 2016; Pappas *et al.*, 2015, 2016; Pérez-Hedo *et al.*, 2015a, 2015b). The activity of zoophytophagous insects such as mirid predators induces a physiological response in plants (Kessler and Baldwin, 2004; Halitschke *et al.*, 2011). Pérez-Hedo *et al.*, (2015b) demonstrated that the feeding activity of *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) activates the abscisic acid (ABA) and jasmonate acid (JA) pathways in tomato plants, which makes them less attractive to the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) and more attractive to the whitefly parasitoid *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae). In addition, herbivore-induced plant volatiles (HIPVs) emitted by N. tenuis damaged plants can induce plant defenses in neighboring undamaged plants via the JA pathway, resulting in parasitoid attraction. The induction of a plant response by a mirid predator may be species dependent (Perez-Hedo *et al.*, 2015b). Females of *N. tenuis*, *Macrolophus pygmaeus* (Rambur) and *Dicyphus maroccanus* Wagner (Hemiptera: Miridae) differ in their ability to induce defensive responses in tomato plants, resulting in varying degrees of plant attractiveness to pests and natural enemies. Tomato plants punctured by *N. tenuis* were less attractive to the whitefly *B. tabaci* and the lepidopteran *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). In contrast, tomato plants exposed to *M. pygmaeus* and *D. maroccanus* were not able to repel *B. tabaci* and, interestingly, became more attractive to *T. absoluta*.

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These previous results highlighted that mirid predators trigger synthesis of HIPVs, which make plants less attractive to herbivores, attract natural enemies and induce defenses in neighboring plants (Fig. 7.5.1). In this work, the volatile emissions from intact and *M. pygmaeus*- and *N. tenuis*-punctured tomato plants using a solid phase microextraction technique combined with gas chromatography-mass spectrometry was characterized, assuming that the volatile blend released is specific for a particular insect-plant system. Ten HIPV's clearly stood out as major differential peaks on the chromatogram. In general, there was a tendency such that *N. tenuis*-punctured plants emitted more volatiles than *M. pygmaeus*-punctured plants, and the latter emitted more volatiles than did the intact plants. When identified, the role of each HIPV in the repellence and/or attraction of two key tomato pests, *B. tabaci* and *T. absoluta*, and to one parasitoid, *E. formosa*, were evaluated.

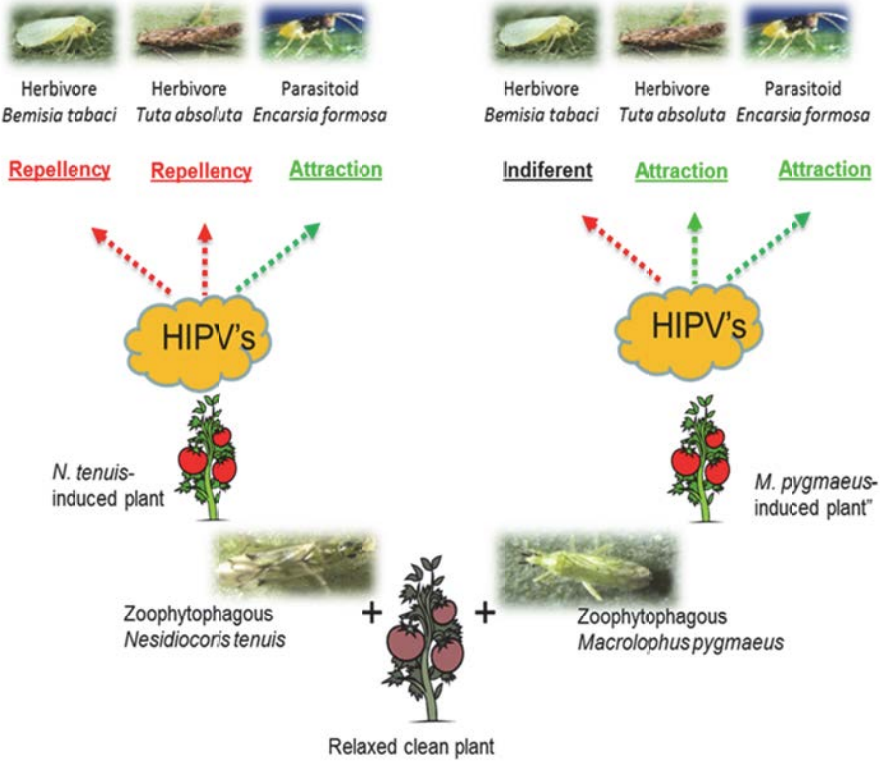

Fig. 7.5.1. A conceptual model of how HIPV's triggered by the zoophytophagous predators *Nesidiocoris tenuis* (left) and *Macrolophus pygmaeus* (right) differ in their ability to induce defensive responses in tomato plants, resulting in varying degrees of plant attractiveness to pests and natural enemies (Adapted from Pérez-Hedo *et al.* 2015a,b).

Our results show that the effectiveness attributed to predatory mirids in pest management is due not only to their predatory behavior but also to their herbivory which, as demonstrated in our work, could modulate pest and natural plant enemy locations, since tomato plants release a blend of volatile compounds in response to their activity. These results could serve as a basis for future development in plant protection from both top-down approaches (i.e., dispenser developments) and bottom-up approaches (i.e., plant breeding).

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Session 8: Weed and Arthropod Biological Control: Mutual Benefits and Challenges

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8.1 Arthropod and Weed Biological Control: Mutual Benefits and Common Challenges

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In this discussion paper we will first compare classical and augmentative biological control of arthropods and weeds and then review current challenges and identify potential mutual benefits and synergies between these two disciplines.

Classical biological control (CBC) of both arthropods and weeds first started at the end of the 19th century. CBC has been used more extensively for the control of insect pests than for weeds, with more introductions against more pests in more countries/islands and slightly more introductions and agent species released per pest (Table 8.1.1). However, the rate of successful establishment is nearly double for weed biological control releases and the proportion of agents with (some) impact on the target organisms also appears to be higher (Table 8.1.1).

Table 8.1.1. Comparison of classical biological control of insect pests and weeds.

	Insect pests[1]		Weeds[2]	
No. introductions / releases	6158		1555	
No. or % of establishments	2084	34%	982	63%
No. of pest/weed species	588	10.5 releases/pest	175	8.9 releases/weed
No. of agent species	2171	4.1 agents/pest	468	2.7 agents/weed
No. of countries & islands	203		90	
No. and % of successful agents	620[3]	30%	332[4]	52%
No. non-target species typically tested	10-30		50-150	

[1]Data through 2010, from the BIOCAT database (Cock *et al.*, 2016), on the basis that where a biological control agent is sourced from more than one country, each source country counts as a separate introduction; [2]Data summarized from Winston *et al.* (2014); [3]Substantial to complete control (see Cock *et al.*, 2016); [4]Refers to the number of established agents that caused medium/variable or heavy impact in at least one release.

Host-specificity studies as part of CBC of weeds were already being done as early as 1902 (van Wilgen *et al.*, 2013), but throughout most of the first half of the 20th century, either no host-specificity tests or only no-choice starvation tests with crop species were conducted in weed CBC. This started to change from the 1960s onwards with the development of new experimental designs and testing of closely related plant species. In

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arthropod biological control, agents were mostly selected based on their killing power and little/no emphasis was placed on potential non-target attack. This only started changing from the 1990s onwards, following reports of negative environmental impacts. Today, many more non-target species are tested pre-release in CBC of weeds compared to arthropods. This is because weed biological control is more scrutinized when it comes to potential non-target attack, especially of economically important or native plant species, necessitating a larger number of non-target species to be tested (Table 8.1.1). In addition, three trophic levels need to be considered in arthropod biological control experiments which limit the number of non-target species that can realistically be tested.

Overall, CBC of arthropods has been somewhat behind CBC of weeds in terms of developing methods to prioritize safe and effective agents and select non-targets, and the development of elements of modern host specificity testing in CBC of arthropods has benefited from the knowhow developed in CBC of weeds (e.g., Bigler *et al*., 2006).

The augmentative biological control (ABC) approach has also been used more extensively for the control of arthropods than for weeds. While 258 active substances and organisms are registered worldwide for use as biological control agents for arthropod pests (BCPC, 2017), only 13 different fungi and bacteria have been developed into bioherbicides for inundative weed control (Winston *et al.*, 2014), and we are not aware of any macro-organism registered for the control of weeds (but see commercially available insects, CBC agents or accidentally introduced species, in the U.S.). We believe that several factors have led to this situation. First, while the application of herbicides has almost completely dominated weed management until the 1990s, warnings about the consequences of the domination of chemical insecticides in pest control, including human health, resistance, resurgence of primary and secondary pests and environmental concerns, raised the popularity of an integrated control concept in arthropod control in the 1960s (Kogan, 1998). Second, the higher level of specificity required for weed biological control is likely to have contributed to the low number of ABC solutions against weeds. For arthropod biocontrol it may be sufficient for an agent to be specific at the family or even order level (e.g., Trichogramma spp., Bt, nematodes etc.), making it possible to release the same agent against a variety of pests, while this would not be acceptable for organisms applied for weed control. And third, cheap mass production is more challenging for specialist herbivores than for arthropod ABC agents, particularly oligophagous predators. Consequently, the development and integration of ABC methods with other management techniques is much further advanced and much more widely used for the control of arthropod pests than for weeds (but see Lym, 2005).

However, there is increasing concern that herbicide resistant weeds are rapidly evolving and spreading (Harker and O'Donovan, 2013), and that herbicides are a major contaminant of surface- and groundwater and contribute to a loss of farmland biodiversity and of the associated ecosystem services. In the EU, many of the currently used herbicides will likely be withdrawn from the market in the near future, increasing the need for a more integrated weed management approach that includes ABC. We believe that particular scope exists to develop *native* natural enemies as a component of integrated weed management (Valenti *et al*., 1999; Cordeau *et al*., 2016). Currently, 44 native organisms have been redistributed and augmented to control native weeds worldwide (Winston *et al*., 2014), but none on such a large scale and so successfully as in arthropod ABC. The experience with existing IPM strategies and the support of ABC companies will therefore be crucial for the further development and implementation of ABC for weeds and integrated weed management strategies. The probably most important potential mutual benefit for weed and arthropod biological control, is to build and offer truly integrated systems, that incorporate weed control, including biological weed control, in existing IPM systems.

However, challenges may limit uptake, such as reliability, effectiveness, speed of action, costs, shelf-life, and the fact that integrated / biological approaches may be more complicated and time consuming to apply, especially in comparison with chemical pesticides (Harker and O'Donovan, 2013; van Lenteren, 2012). In addition, increasing risk aversion and consequently more stringent regulations as well as limitations to access genetic resources due to the implementation of the Nagoya Protocol have hampered the use and development of CBC in particular, but also ABC, in recent years (van Lenteren, 2012; Cock *et al.,* 2016). Arthropod and weed biocontrol practitioners should work together to face these challenges as is already being done in respect to the Nagoya Protocol with the development of common best practices (Mason *et al*., 2017; Barratt *et al*., this volume, 4.3).

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Session 9: Maximizing Opportunities for Biological Control in Asia's Rapidly Changing Agro-environments

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9.1 From Molecule to Landscape - Integrating Molecular Biology and Landscape Ecology to Open New Opportunities for Biological Control in East Asia

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At the time of the 2017 ISBCA, biological control has never been at a more exciting stage of development, and the need for biological control has never been greater. This paper will explore how the recent advances in two particular fields open great opportunities to both understand fundamental aspects of biology that underpin our discipline as well as to deliver more effective solutions to end-users. First, the rapidly falling cost of DNA sequencing and capacity for bioinformatics analyses mean that genomic data-rich studies of agents and functionally related species are increasingly feasible and powerful. Second, theoretical advances in spatial ecology are complemented by unmanned aerial vehicles (UAVs or ‘drones’) as platforms for advanced remote, hyperspectral imaging. These high-tech approaches will allow biological control to more consistently achieve end-user benefits such as in recent work in Asian rice (Gurr *et al.*, 2016).

Traditionally, biological control research effort has been at the level of whole organisms, the agents and their targets. A crude illustration of this is that a search of Web of Science for ladybird AND biological control returns more than 1400 articles dating back over a century to work in Arizona (Morrill, 1913). In more recent decades, biological control researchers have explored how manipulation of the environment, usually at the field scale, can promote natural enemy activity. This is reflected by a search of Web of Science using habitat manipulation AND biological control which finds 137 papers dating back less than 40 years to the work by Altieri and Whitcomb (1979). Work at the larger spatial scale dates back over a similar period. Web of Science records for landscape AND biological control number 1500 and range back to the early 1970s (Brewer, 1972). Perhaps surprisingly, a search for molecular AND biological control finds over 2500 papers dating back to the early 1930s including work on chemical stimulation of parasitoid ovipositors (Dethier, 1947). Even a search for genomic AND biological control finds well over 1000 papers extending back to 1982 work on baculoviruses (Huang *et al.*, 1982). Accordingly, biological control research has long been attuned to the relevance of the extreme levels of biological organisations (i.e., from molecule to landscape) (Fig. 9.1.1). The exciting prospect now awaiting the field is that technological advances now provide far greater power, accessibility and affordability.

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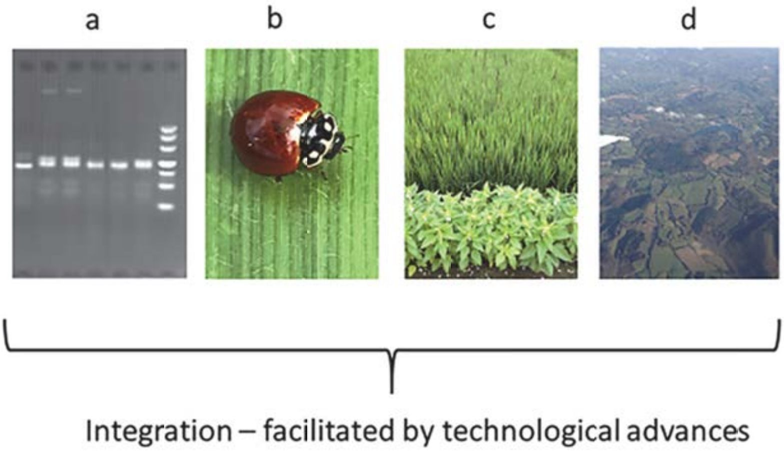

Fig. 9.1.1. Levels of biological organisation relevant to biological control. Traditionally, research effort has been at the level of whole organisms (agents), panel **b**; work in more recent decades has often used local scale habitat manipulation to promote biological control (with sesame nectar plants, for example), panel **c**; or considered the influence of the wider landscape, panel **d**; and at the opposite (molecular) extreme, panel **a**.

At the molecular and genomic level, the rapidly declining cost of analyses, especially sequencing (Metzker, 2010) is providing unprecedented power to understand phenomena such as genetic control of traits and adaptation to environmental conditions in biological control agents and targets. At the other extreme, advances in imaging, remote sensing and – especially – UAVs have been rapid in the last decade. UAVs can be purchased cheaply via the Internet and are already being assessed for surveillance, monitoring and even release of biological control agents (Faithpraise *et al.*, 2015). Hyperspectral imaging systems now offer scope for remote sensing of pests (Nansen, 2016). Combining imaging technologies with UAVs could allow efficiencies in inundative biological control programs by making it fast, cheap and easy to release appropriate numbers of agents in positions within the crop only where control is needed. As the cost of robotic technologies declines, it is likely to be become possible to use UAV-mounted robotic equipment to efficiently sample pests, plants or agents in a spatially explicit manner from the landscape. These could then be processed for various types of analyses – including genomic – to assess factors such as insecticide resistance genes in the pest (indicating a particular need for biological control) and the nutritional status of parasitoids (indicating the need for more nectar plants). Ultimately, genomic analyses could be done on such samples to build comprehensive landscape meta-genomic maps of biological control agents, targets, and the microbes and plants associated with them in the landscape, and thereby optimise control of pests. This capacity will have great utility in allowing global agriculture to meet human needs, but especially so in Asia where high population densities and growth rates make the challenge especially great.

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9.2 Phyto-pathogens and Soil Nutrients Shape Biological Control of Invasive Mealybugs in Asia's Cassava Crops

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Over the past decade, Southeast Asia has been subject to successive invasions by different species of mealybugs (Homoptera: Pseudococcidae). These include (1) the cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero), a Neotropical parthenogenetic, oligophagous herbivore with broad climatic adaptability; (2) the papaya mealybug (*Paracoccus marginatus* Williams and Granara de Willink) a Nearctic sexual, polyphagous feeder; and (3) *Pseudococcus jackbeardsleyi* Gimpel & Miller, a Neotropical polyphagous species. Though all above species have colonized vast swathes of South East Asia's countryside, they have particularly affected cassava; a major food, feed, starch and bio-energy crop that's cultivated on >4 million ha regionally. Locally, cassava is grown in highly-heterogeneous settings – ranging from large-scale, intensified monocultures in the fertile lowlands of Vietnam and Thailand, to smallholder-managed shifting cultivation on degraded lands in the region's uplands. As a consequence, the edaphic conditions under which cassava is cultivated are highly variable. Though soil variables (e.g., texture, fertility, organic matter content) are thought to affect overall performance of the above mealybug species and their associated (endemic, or introduced) natural enemies, only scant information is available on these kinds of trophic dynamic processes, particularly in tropical agro-ecosystems. As a consequence, biological practitioners face important challenges to make reliable, context-specific inferences on pest pressure, natural enemy performance and efficacy of biological control.

In this study, we assess to what extent soil parameters determine the relative success of the three above mealybug species and the degree of *P. manihoti* biological control. We combine potted plant fertilizer trials (for *P. manihoti*) under screen-house conditions in central Vietnam, with general pest surveys (for all three mealybugs) in 65 cassava fields across southern Vietnam, Laos and Cambodia. In the potted plant trials, we examine the extent to which single-element additions of N and K fertilizer impact different life-history parameters of *P. manihoti* and its encyrtid parasitoid *Anagyrus lopezi* De Santis. For the survey, fields were chosen of similar size and age, but with varying soil fertility. Fields were 6-9 months old, were managed by individual farmers using standard crop and soil management practices, and were located in the countries' primary cassava-growing regions. In each field, a total of five soil samples were collected and a total of 13 different parameters were recorded, including N, P, organic carbon and multiple soil texture measures, amongst others. Furthermore, mealybug infestation levels were assessed within each field, and *P. manihoti* parasitism rates (by the introduced encyrtid *A. lopezi*) were determined in a subset of those. Lastly, we recorded field-level incidence of cassava

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witches broom disease (CWB), a phytoplasma disease that is common across the region. Field-level abundance of each mealybug species was mapped along a soil fertility gradient, and also contrasted with site-specific measures of parasitism in the case of *P. manihoti*.

Pot trials reveal strong bottom-up effects for the *P. manihoti* system, such that nitrogen and potassium additions are propagated through to higher trophic levels and increase development and fitness of its *A. lopezi* parasitoid. Field surveys on the other hand indicate that mealybug performance is highly species-specific and context-dependent. For example, field-level abundance of *P. jackbeardsleyi* and *P. marginatus* is related to aggregative measures of soil fertility, soil texture and CWB disease incidence. While, for *P. manihoti*, in-field abundance is largely associated with soil texture (i.e., silt content) or water-holding capacity. Principal component regression suggests that *P. manihoti* and *P. marginatus* are disproportionately favored in low-fertility contexts, while *P. jackbeardsleyi* prospers in settings with high organic carbon and phosphorus.

Our work thus shows that (1) potted plant fertilizer trials reveal the extent to which single-element additions of nitrogen (N) and potassium (K) benefit *P. manihoti* growth, while increasing fitness of its parasitoid, *A. lopezi*; (2) field-level abundance of all three invasive species does vary greatly along a soil fertility gradient, with different mealybug species responding to particular soil fertility measures; (3) top-down forces (i.e., parasitism by the recently-introduced natural enemy *A. lopezi*) shift in importance between varying soil fertility contexts.

In conclusion, our work reveals how trophic regulatory forces (i.e., resource-mediated drivers, parasitoid attack) greatly affect performance of three invasive mealybugs in Asia's cassava cropping systems. Important species-specific and context-dependent patterns can be detected, with *P. manihoti* biological control being strongly dependent upon site fertility and soil texture. Plant nutrient availability, N in particular, appears to affect multiple trophic levels –i.e., both herbivores and parasitoids alike. Soil fertility and plant quality variables, either singly or combined, should thus be taken into consideration when developing invasive species management and biological control programs. Our findings from smallholder systems in the tropics underline that agricultural pest management need to be addressed in a holistic and integrated fashion, and that it is essential for biological control practitioners to engage in interdisciplinary communication and collaboration.

9.3 Recent Change of Biocontrol Services in Cotton Agro-ecosystem of Northern China

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Vast plantings of transgenic crops producing insecticidal proteins from the bacterium *Bacillus thuringiensis* Berliner (Bacillaceae) (Bt) have helped to control several major insect pests and reduce insecticide sprays. Because broad-spectrum insecticide sprays kill arthropod natural enemies that provide biological control of pests, the reduction in insecticide sprays associated with Bt crops could enhance biocontrol services. However, this hypothesis has not been tested in terms of long-term landscape-level impacts.

Predators and cotton aphid were sampled from 2001 to 2011 in Bt and non-Bt cotton plots at the Langfang Experiment Station in the Hebei province, China. No significant differences were found for predator and aphid abundances between Bt cotton and non-Bt cotton with similar management methods, i.e. without application of insecticides. However, predator abundance was significantly lower and aphid abundance was significantly higher in plots treated with insecticides for cotton bollworm management vs. insecticide-free plots. Thereafter, insecticide-induced aphid resurgence usually occurs with widespread insecticide applications. Based on data from 1990 to 2010 at 36 sites in six provinces of northern China, we showed a marked increase in abundance of three types of generalist arthropod predators (ladybirds, lacewings, and spiders) and decreased abundance of aphid pests associated with wide-scale adoption of Bt cotton and reduced insecticide sprays in this crop. Predator population levels and number of insecticide sprays were respectively positively and negatively related to Bt cotton planting proportions, and indicated the effect of its wide-scale adoption on the predator population trend. Additionally, increasing generalist predator populations were correlated with decreasing aphid abundance in Northern China. During the three main periods studied, i.e. without Bt cotton, with <90% and >90% of Bt cotton planting in the landscapes, aphid populations significantly decreased. In addition, aphid population level was negatively related to Bt cotton planting proportions. Predator abundance was also monitored from 2001 to 2011 in three neighboring crops; maize, peanut and soybean at the Langfang Experiment Station. We also found evidence that the predators might provide additional biocontrol services spilling over from Bt cotton fields onto neighboring crops (maize, peanut and soybean). Our work extends results from general studies evaluating ecological effects of Bt crops by demonstrating that such crops can promote biocontrol services in agricultural landscapes.

During 2013-2015, we assessed the effects of landscape composition on predator abundances in cotton fields in northern China. Principal component analysis and an adjusted Akaike's Information Criterion analysis of the data from 41 study sites indicated that landscape composition and diversity affected the abundance of predators on cotton.

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More specifically, landscape with a high proportion of some small crops (such as peanut, soybean, sweet potato, vegetable crops and fruit trees) other than major crops (cotton and maize) generally supported high abundance of predators in cotton field. Landscape diversity enhanced the aphid parasitoids abundance and their parasitism on cotton at large spatial scales (1-2 km radius) across all 35 study sites. The proportion of mummies ranged from 0-53.5% with a mean value of 12.5%, which was supported with a higher proportion of other host crops than cotton and maize (0.5-2 km) and urban habitats (1-2 km), and was negative correlated with the proportion of cotton and maize. It indicates that predator and parasitoid abundances and their associated biocontrol services may be decreasing with great increase of maize planting in northern China.

These studies provide a primary understanding of the relationship between cropping pattern and the community of natural enemies and their ecosystem services for pest aphid management in cotton in changing small farming systems, especially the commercialization of Bt cotton and increasing of maize planting in China.

9.4 Know your Enemies: Suppression of *Plutella xylostella* and *Crocidolomia pavonana* by Different Predators in West Java, Indonesia

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Plutella xylostella L. (Lepidoptera: Plutellidae) and *Crocidolomia pavonana* F. (Lepidoptera: Crambidae) are serious, co-occurring pests of *Brassica* crops in the highlands of West Java, Indonesia. Prior to the introduction of synthetic pesticides in the region, *C. pavonana* was considered a more serious pest than *P. xylostella* (Ankersmit, 1953), although *P. xylostella* has long been considered the more serious pest at drier times of the year (Vos, 1953). Together, the pests often result in complete crop loss in West Java, particularly during the dry season (Sastrosiswojo and Setiawati, 1992).

Diadegma semiclausum Hellén (Hymenoptera: Ichneumonidae), a *P. xylostella* parasitoid of European origin, was introduced from New Zealand in 1950 (Vos, 1953) and it successfully established in highland regions, including West Java (Sastrosiswojo and Sastrodihardjo, 1986). Parasitoids do not provide effective control of *C. pavonana* anywhere in the world (Uelese *et al*., 2014) and in West Java, two endemic larval parasitoids that attack the pest, *Eriborus argenteopilosus* Cam. (Hymenoptera: Ichneumonidae) and *Sturmia inconspicuoides* Bar. (Diptera: Tachinidae), only occur at very low levels (Sastrosiswojo and Setiawati, 1992).

Despite the lack of effective parasitoids of *C. pavonana* in Indonesia, Shepard & Schellhorn (1997) suggested that if no chemical insecticides were applied to crops then predators would most likely become key mortality factors for *C. pavonana.* However, the empirical evidence to support the claim that predation of this pest is important is lacking. Similarly, despite the extensive studies that have been conducted on biological control of *P. xylostella* worldwide (Furlong *et al.,* 2013; Li *et al.,* 2016), the majority of studies do not consider the impact of predators on pest populations and those that do frequently fail to assess the impact of predators appropriately (Furlong and Zalucki, 2010). Typically, studies of arthropod predation report the abundance and diversity of predator species that are commonly found in *Brassica* crops (Miranda *et al.,* 2011; Sastrosiswojo *et al*., 2004) but they do not progress to develop an understanding of the ecological impact of predators on pest populations so that contributions to biological control can be evaluated.

Quantifying the impact of natural enemies on target pest populations is essential if these ecosystem services are to be used for pest management decision-making (Furlong and Zalucki, 2010). Once the impact of natural enemies on a pest population has been demonstrated, the next step is to determine the key mortality factors through life table construction; this also provides vital information on species interactions and the ecological

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role of different natural enemies. The impact of predators on pest populations is difficult to demonstrate. Predators often leave no remains of their prey and attributing mortality to a given group of predators is difficult without the means to detect evidence of specific predation events; this requires experimental manipulation of both pest and natural enemy populations (Furlong and Zalucki, 2010). These approaches then need to be combined with a reliable method to determine which predators consume target pests to evaluate their importance in pest suppression; for example, visual observation to detect and document incidents of predation directly, or combined with molecular analyses to detect the presence of prey within the predator (Weber and Lundgren, 2009).

Although considerable advances have been made in the development of DNA-based molecular techniques to detect the remains of insect prey within the guts of predatory arthropods, the methods have rarely been applied in conjunction with field experiments that measure the impact of natural enemies on pest/ prey populations (Furlong, 2015). In this study, field experiments used a combination of ecological (natural enemy exclusion techniques and life table construction) and DNA-based molecular methods to quantify the impact of different predatory arthropods on *P. xylostella* and *C. pavonana* populations in *Brassica* vegetable crops in West Java. Prior to these quantitative studies, the specificity of previously designed primer sequences for *P. xylostella* and *C. pavonana* mtCO1 DNA (Furlong *et al.*, 2014) was confirmed by testing them against a wide range of herbivores and predatory arthropods collected from in West Java.

In a series of field studies that simultaneously investigated the impact of the natural enemy complex on *P. xylostella* and *C. pavonana* populations (Fig. 9.4.1), the endemic predator complex and *D. semiclausum* consistently suppressed *P. xylostella* populations, but predators had a greater impact than the parasitoid. The proportion of the original *P. xylostella* cohort recovered from natural enemy exclusion cages (95% and 89%) was significantly greater than the proportion recovered from open cages (2% and 1%) in both the first ($t= 15.40$, $P< 0.001$) and second $t= 10.43$, $P< 0.001$) experiments. No parasitoids were reared from *P. xylostella* that developed in the exclusion cages in either experiment. However, *D. semiclausum* was reared from *P. xylostella* developing in open cages and *D. semiclausum* marginal parasitism rates of 0.33 and 0.63 were recorded in the first and second experiments respectively. In both experiments, the net reproductive rates (R_0) of the *P. xylostella* cohorts in the presence of natural enemies were lower (0.3 and 0.5 respectively) than those in the absence of natural enemies (56.3 and 52.8 respectively).

In the first experiment, the proportion of the original *C. pavonana* cohort recovered from natural enemy exclusion cages (59%) was significantly greater than the proportion recovered from open cages (15%) ($t= 2.67$, $P< 0.014$) but there was no significant difference between recovery rates from exclusion (54%) and open cages (38%) in the second experiment ($t= 1.07$, $P= 0.297$). No parasitoids were reared from any *C. pavonana* larvae. In the first experiment, R_0 of the cohort reared in open cages was 14.9, considerably lower than the R_0 of the cohort reared in exclusion cages (69.0). However, in the second experiment, R_0 of the cohorts reared in open (61.2) and exclusion cages ($R_0 = 79.3$) were very similar.

Fig. 9.4.1. Natural enemy exclusion and open cages in an experimental cabbage field at the Indonesian Vegetable Research Institute, Lembang, West Java. Studies simultaneously investigated the impact of the endemic natural enemy complex on experimental cohorts of *Plutella xylostella* and *Crocidolomia pavonana*. Bamboo canes mark the location of pitfall traps.

Most foliar-dwelling predators collected by destructively sampling in-field plants during the natural enemy exclusion studies were predatory insects and they accounted for 58% of all arthropod predators (n = 309) collected from foliage. The most abundant predatory insects found foraging on plants were Syrphidae (50% of 175 predatory insects caught; 10% contained *P. xylostella* mtCO1 DNA and 17% *C. pavonana* mtCO1 DNA). The next most abundant predatory insects collected on foliage were *Menochilus sp.* (n = 36; 8% contained *P. xylostella* mtCO1 DNA, 11% contained *C. pavonana* mtCO1 DNA but none contained mtCO1 DNA of both species;), Staphylinidae (n = 17; 13 *Paederus* sp. (77% contained *C. pavonana* mtCO1 DNA but none contained *P. xylostella* mtCO1 DNA) and 4 unidentified individuals (all contained *P. xylostella* mtCO1 DNA, but none contained *C. pavonana* mtCO1 DNA) and Miridae (n = 11; 1 contained *P. xylostella* mtCO1 DNA and 2 contained *C. pavonana* mtCO1 DNA). The most abundant spiders collected by destructive sampling of plants were Araneidae (57% of the 134 spiders collected), followed by Gnaphosidae (25%) and Clubionidae (≈5%), Linyphiidae (≈5%) and Theridiidae (≈5%). *Plutella xylostella* mtCO1 DNA was found in 50% of the Clubionidae, and 11-17% of the Gnaphosidae, Aranaeidae, Linyphiidae and Theridiidae. Only Araneidae (7%) and Gnaphosidae (3%) contained *C. pavonana* mtCO1 DNA and no spiders contained mtCO1 DNA of both prey species.

Most epigeal predators caught in pitfall traps set to capture predators for gut-content analysis were predatory insects (75% of the 182 captured by this method). Formicidae were the most abundant predatory insects caught in the pitfall traps (80% of 137 predatory insects caught). Four genera of Formicidae were collected, *Pheidole sp., Hypoponera sp., Crematogaster sp.* and *Myrmecina sp. Pheidole sp.* was the most abundant (66% of 109;

54% contained *P. xylostella* mtCO1 DNA, 10% contained *C. pavonana* mtCO1 DNA and 19% contained mtCO1 DNA of both *P. xylostella* and *C. pavonana*. *Hypoponera sp.* was the next most abundant (29% of 109, 47% contained *C. pavonana* mtCO1 DNA but only 3% contained *P. xylostella* mtCO1 DNA).

The most abundant spiders caught in the pitfall traps were Lycosidae (76% of 45 individuals); 29% of these contained *C. pavonana* mtCO1 DNA, 15% contained *P. xylostella* mtCO1 DNA and 3% contained both *P. xylostella* and *C. pavonana* mtCO1 DNA. Araneidae (n = 2), Gnaphosidae (n = 7) and Linyphiidae (n = 1) were also caught in pitfall traps. One Gnaphosidae contained *C. pavonana* mtCO1 DNA, another contained both *C. pavonana* and mtCO1 DNA and the single Linyphiidae caught contained *P. xylostella* mtCO1 DNA; neither of the Araneidae contained prey DNA.

Foliar dwelling spiders appear to be more important predators of *P. xylostella* than of *C. pavonana*, while epigeal Lycosidae are more likely to have preyed upon *C. pavonana*. This possibly reflects the increased likelihood that these predominantly soil surface dwelling predators will encounter *C. pavonana*, which pupates in the soil, over *P. xylostella* which typically completes its lifecycle on its host plant. Similarly, *Paederus* sp. was more likely to have preyed upon *C. pavonana*, while the unidentified species of staphylinid only preyed upon *P. xylostella*. Of the ants, *Hypoponera sp.* was more likely to have preyed upon *C. pavonana* while *Pheidole sp.* was more likely to have preyed upon *P. xylostella*. Whether these differences represent distinct feeding preferences between these species or are simply the result of patchy distributions of the predators within the experimental field, requires further investigation.

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9.5 Biological Control in Vegetable *Brassica* Pest Management in Tropical Asia: Where Do We Currently Stand?

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Vegetable brassicas including Chinese cabbage, *Brassica rapa* L. var. *pekinensis*, pak-choi, *B. rapa* var. *chinensis*, choisum, *B. rapa* var. *parachinensis,* and Chinese kale, *B. oleracea* L. var. *alboglabra* (Brassicaceae), are among the most important vegetables in Asia. Production of vegetable brassicas is constrained by a plethora of insect pests including diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), cabbage head caterpillar, *Crocidolomia pavonana* Fabricius (Lepidoptera: Crambidae), cabbage web worm, *Hellula undalis* Fab. (Lepidoptera: Crambidae), imported cabbage worm, *Pieris rapae* L. (Lepidoptera: Pieridae), aphids, *Myzus persicae* Sulzer, *Lipaphis erysimi* Kaltenbach, and *Brevicoryne brassicae* L. (Hemiptera: Aphididae) and flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae) in tropical Asia. However, *P. xylostella* can be brought under reasonable control with the introduced larval parasitoid, *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) in highlands. Performance of *D. semiclausum* is complemented by the pupal parasitoid, *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae), another classical bio-control agent in the Asian highlands. *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae), the predominant larval parasitoid in lowland brassica production systems offer significant control of *P. xylostella*. Hence, World Vegetable Center (WorldVeg) took a lead in expanding the introduction of these bio-control agents against *P. xylostella* in tropical Asia for the past four decades. However, absence of effective bio-control agents targeting the secondary lepidopterans still triggers the brassica producers to rely more on chemical pesticides, which actually disrupts the biological control of *P. xylostella* and thus leading to the resurence of this pest (Talekar, 2004). In addition, absence of an effective pupal parasitoid, which can complement *C. plutellae* also leads to pesticide overuse on brassicas in Asian lowlands. Hence, there is an urgent need to expand the bio-control options against major insect pests of vegetable brassicas in Asia.

Identification of a heat tolerant strain of *D. semiclausum* in Syrian lowlands (Kadirvel *et al.,* 2011) has opened up new avenues for *P. xylostella* management in lowland vegetable brassica production in rest of Asia. This strain was collected from the lowland areas of Homs, Syria (about 203-487 m above sea level) and it thrived well when the parasitized *P. xylostella* larvae were reared at day and night temperatures of 35°C and 20°C, respectively. Similarly, identification of an arrhenotokous population of the generalist egg parasitoid,

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Trichogramma chilonis Ishii (Hymenoptera: Trichogrammatidae) parasitizing *C. pavonana* egg masses to a maximum extent of 87% in Samoa (Uelese *et al.*, 2014) enhances the potential for biological control of *C. pavonana* elsewhere.

During 2014-2016, we conducted random and sporadic surveys in vegetable brassica fields in Taiwan and Vietnam to identify the occurrence of parasitoids on secondary lepidopterans and the sucking pests. The results from our surveys confirmed the larval parasitoid, *Cotesia glomerata* L. (Hymenoptera: Braconidae) (Fig. 9.5.1) and a pupal parasitoid, *Pteromalus puparum* L. (Hymenoptera: Pteromalidae) (Fig. 9.5.2) as major parasitoids of *P. rapae*. Similarly, *Diaeretiella rapae* McIntosh (Hymenoptera: Braconidae) was found to be the major parasitoid of aphids on brassicas in Vietnam. Although *D. rapae* parasitises about 98 species of aphids infesting more than 180 plant species belonging to 43 plant families distributed in 87 countries, the most preferred host insects are brassica aphids including *B. brassicae*, *M. persicae* and *L. erysimi* (Singh and Singh, 2015). Hence, these natural enemies increase the scope of brassica bio-control programs in tropical Asia. Thus, conservation and/or augmentation of various bio-control agents against *P. xylostella* as well as secondary lepdiopterans on vegetable brassicas in tropical Asia have become imperative to reduce the pesticide misuse. In addition, bio-pesticides including *Bacillus thuringiensis* (Srinivasan, 2012) and sex pheromone lures (Zhao *et al.,* 2011) can enhance the performance of natural enemies and thus offering integrated pest management options for safer brassica production in tropical Asia.

Fig. 9.5.1. Cocoons of *Cotesia glomerata* on *Pieris rapae* cadaver.

Fig. 9.5.2. Adults of *Pteromalus puparum* parasitizing *Pieris rapae* pupa.

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Session 10: Biological Control Based Integrated Pest Management: Does it Work?

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10.1 Successful Integrated Pest Management With Biological Control: Case of the Diamondback Moth in Malaysia

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The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), has been recorded in Malaysia since 1925 (Ho, 1965). It became an important pest of crucifers grown in the highlands from 1941, causing damage to major brassicas, such as cabbage. Historically, the control of this pest has been with insecticides that are used indiscriminately by farmers. This has led to the development of insecticide resistance to almost all classes of pesticides including *Bacillus thuringiensis*-based products (Syed, 1992). Between 1985 and 1989, triggered by media reports highlighting consumer concerns on health due to farmer's over-dosing highland vegetables with pesticides to control DBM, the scientists in the Malaysian Agricultural Research and Development Insitute (MARDI) led studies on an Integrated Pest Management (IPM) program in the highlands (Cameron Highlands) on this pest. The key component in this IPM program was the use of biological control agents (parasitoids) based on exploitation of the indigenous *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) and enhanced by exotic parasitoids such as the larval parasitoid, *Diadegma semiclausum* (Hellen) and the pupal parasitoid, *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae).

The IPM program for DBM was ecologically-intensive, underpinned by several studies that included crop and life table analysis and dynamics of key pests, impact assessments of natural enemies on prey and their food sources, development of bio-based tactical components and formulation of dynamic economic threshold levels based on sampling techniques (Lim *et al.*, 1986; Loke *et al.*, 1997; Sivapragasam *et al.*, 1985). Amongst the quantitative studies conducted were the competitive host searching behaviour of the parasitoids based on their functional responses to host larva as described by the Random Parasite Equation (Rogers, 1972). The key parameters that described these responses were the searching rate (a') and the handling time (T_h). The data suggested that the exotic *D. semiclausum* (a' = 1.887; T_h = 0.0092 days) had a superior seaching rate and lower handling time when compared with the indigenous *C. plutellae* (a' = 0.813; T_h = 0.0115 days). Laboratory studies on interspecific competition between the two parasitoids revealed that *D. semiclausum* was also intrinsically superior to *C. plutellae* when it has first access to the host larva and *vice versa*.

After the field introduction of the parasitoids in the early 80s, their impact was initially not clear (Ooi, 1992). However, low numbers were seen in unsprayed cabbage areas in the MARDI research plots where the introductions were made. However, Ooi (1992) showed

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that the DBM population in 1989 was significantly reduced as compared with that in 1977 and this was attributed to the impact of the parasitoids especially due to *D. semiclausum* (Fig. 10.1.1; Ooi, 1992). Syed *et al.* (1997) also recorded a similar phenomenon in their observations.

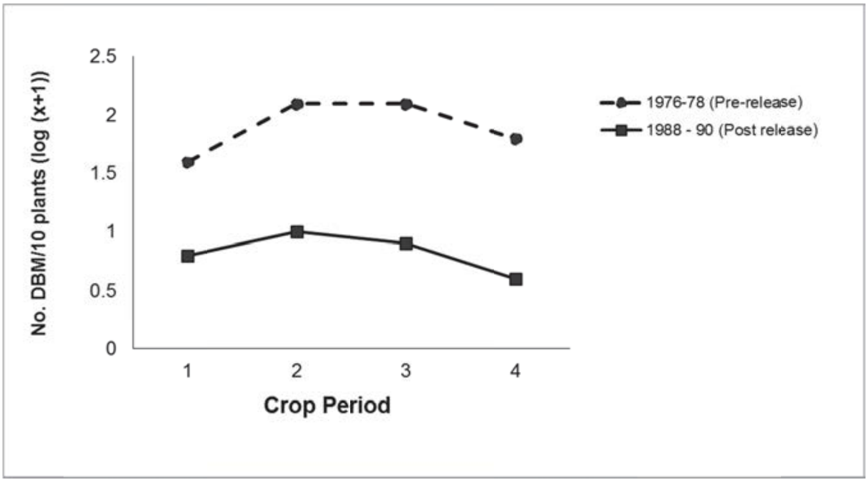

Fig. 10.1.1. Number of diamondback moth larva on cabbage plants recorded during various crop cycles at the pre-release and post release periods of exotic parasitoids.

Fortunately, despite the high insecticide use by farmers, all three major parasitoids were still present in Cameron Highlands as shown in a latest 2011 survey (Table 10.1.1; Sivapragasam *et al.*, 2011). The pupal parasitoid *D. collaris* was, however, not recorded in the Southern zone during the survey. The major parasitoid in the Northern and Southern zones was still *C. plutellae* which was also reported by Syed *et al.* (1997). *D. semiclausum* was recorded in all the zones albeit in lower numbers than those recorded by Syed *et al.* (1997).

Table 10.1.1. Incidence of diamondback moth and its parasitoids in Cameron Highlands (total number per 30 plants).

Zone in Cameron Highlands[1]	DBM larva+pupa	Parasitoid species		
		Diadegma semiclausum	*Cotesia plutellae*	*Diadromus collaris*
Northern (Kampung Raja) (1,000 -1,100 m asl)	47.5	28.5	38.5	6.0
Central (Kea Farm, Mensum Valley) (1,400 -1500 m asl)	34.5	13.5	3.0	4.5
Southern (Ringlet) (1,000 – 1,100 m asl)	28.5	12.5	16.0	0.0

[1] asl – above sea level

The conclusion of the biological control based IPM program for DBM in Malaysia (and elsewhere too) points to the fact that biological control works – if given the right conditions and within an IPM framework. In the Malaysian context, these include: (i) Selection and introduction of the exotic parasitoids with the requisite biological and ecological attributes and which complement the indigenous species; (ii) Species that are easy to rear and established mass rearing facilities for periodic field releases of these parasitoids; (iii) Pragmatic action thresholds for initiating bio-pesticide and general pesticide use; and (iv) Strong support from various stakeholders involved in the overall production ecosystem, viz., farmers, extension staffs, policy makers, agro-dealers based on the understanding and committment to the program to ensure its sustainability. For example, the Malaysian government mandated the requirement for the inclusion of information on the impact of a pesticide on the key natural enemies as part of its new pesticide registration process.

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10.2 A Practice of *Trichogramma*-based IPM of Rice Insect Pests

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Insect pests, particularly the striped stem borers *Chilo suppressalis* (Walker), the yellow stem borer *Scirpophaga incertulas* Walker, the leaf roller *Cnaphalocrocis medinalis* Guenée (Lepidoptera: Crambidae), the brown planthopper *Nilaparvata lugens* (Stål), and the white-backed planthopper *Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae), are the most consistent constraining factors in rice production (Jena *et al.*, 2012). Potential *Trichogramma* (Hymenoptera: Trichogrammatidae) strains were collected from the Greater Mekong Subregion (GMS) (Liu *et al.*, 2017), and tested for their suitability for mass production, tolerance to high temperatures typically occurring in the GMS, and performance against the targeted pests to select for the best performing species/strains. To ensure compatibility of released *Trichogramma* with indispensable insecticide application for control of planthoppers, the toxicity and timing of application of insecticides were evaluated. On the basis of these laboratory and field tests, a *Trichogramma*-based IPM was established and demonstrated.

In the tests for suitability for mass production at 25ºC, life history traits differed significantly among the strains and the three *Trichogramma chilonis* Ishii strains and a *T. ostriniae* Pang and Chen strain were of high performance (Guo *et al.*, unpublished data).

When female wasps from the 25ºC cultures were exposed to 34 and 37ºC, the usual ambient air temperatures in the GMS, for a whole generation, negative effects were observed on key life history parameters. Effects on parental females and their offsprings were observed at 34ºC, and these became even more serious at 37ºC. In particular, the two *T. ostrinae* strains were not able to successfully develop to adulthood at all at 37ºC while the *T. chilonis* strains showed offspring adult emergence,but offspring female longevity was only around one day and no parasitism was observed. When the three *T. chilonis* strains were exposed to 37ºC for 4 to 12 h at prepupal and pupal stages, reflecting heat

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shocks that the released *Trichogramma* may experience in the field, adult emergence when the exposure was 12 h was lower than that at 25ºC. Despite this, the three *T. chilonis* strains showed relatively high potential for mass production and field release in the subtropical target region. Our results demonstrate that tests at both rearing and field temperatures are necessary in selection of potential *Trichogramma* strains for an inundative release program where there is significant difference between rearing and field temperatures (Guo et al., unpublished data).

Performance of potential *Trichogramma* strains against the target pests, *C. suppressalis* and *S. incertulas*, was tested in laboratory and field experiments. In laboratory vial tests against *C. suppressalis*, all the *Trichogramma* strains showed higher parasitism rates on 0-24-h eggs than on the two older age groups (24-48 and 48-72 h). Wasp emergence rate was also higher from parasitized 0-24-h *C. suppressalis* eggs. Parasitism rates differed among *Trichogramma* strains, with *T. chilonis* CJ strain showing a significantly higher parasitism rate than any other strains. In field tests, parasitism of sentinel *C. suppressalis* eggs by *Trichogramma* strains released at 50,000, 100,000, and 200,000 wasps per hectare was low, with marginal yet significant differences between strains. The highest parasitism was achieved by *T. chilonis* CJ strain at the high and medium release rates. Hence, it can be concluded that *T. chilonis* CJ strain released at 100,000 wasps per hectare may be a cost-effective control tactic for field releases targeting *C. suppressalis* (Ko *et al.*, 2014).

Against *S. incertulas*, *T. japonicum* and *T. chilonis* were tested for their performance. A laboratory cage test showed that the two species parasitized *S. incertulas* egg masses at 60.0 ±9.1% and 40.7 ±7.1%, respectively, with egg parasitism rates of 15.8 ±22.2% for *T. japonicum* and 2.8 ±5.0% for *T. chilonis*. Emergence rates from parasitized eggs were high for both species (> 95%). In paddy field trials with release rates at 50,000, 100,000 and 200,000 wasps/ha, egg mass parasitism was 9 ±7.7% for *T. japonicum* and 15 ±14.1% for *T. chilonis*, and again only a relatively small fraction of eggs was successfully parasitized. No clear conclusion could be drawn on the most efficient release rate for *S. incertulas* as no significant differences were found among the three release rates. A comparison of field collected and mass reared wasps showed significantly larger body size and ovipositor length in field collected wasps, suggesting potentially higher effectiveness of the released *Trichogramma* on *S. incertulas* eggs after at least one generation (Tang *et al.*, 2017).

Compatibility of released biocontrol agents with other management measures, especially pesticides, has to be considered in any IPM systems. Planthopper-targeting insecticides, pymetrozine, thiamethoxam, buprofezin and nitenpyram, were tested for toxicity to adults and immatures of *T. chilonis* using standard tests described by IOBC (Sterk *et al.*, 1999). In the dry film residue test, all insecticides resulted in more than 90% mortality in wasp adults and were ranked as moderately harmful. Persistent toxicity tests revealed that nitenpyram was short-lived and the other three insecticides were of slightly persistent toxicity to the wasp adults. Insecticides applied on egg, larval and prepupal stages of *T. chilonis* reduced the parasitism rate, but nitenpyram and pymetrozine applied at egg stage, buprofezin and nitenpyram at larval stage, and buprofezin and thiamethoxam at prepupal stage were ranked as harmless. Although insecticide treatment of the three wasp immature stages all reduced wasp emergence, only thiamethoxam applied at larval stage and buprofezin at prepupal stage were categorized as harmful. The data are of significance for IPM programs incorporating inundative release of *Trichogramma* for control of lepidopteran rice pests where there is heavy co-occurrence of planthoppers (Ko *et al.*, 2015).

A *Trichogramma*-based IPM was established and demonstrated, which included the such key elements as monitoring of pests as basis for decision making, release of *Trichogramma* at 3-4 times per season, reduction of chemical insecticide applications and use of bio-pesticides, growing nectar plants on bunds, balanced fertilization, and alternative wetting and drying. The demonstration showed that, while IPM plots received 1.5-2.5 times less pesticide applications than the control, pest incidence was generally similar between IPM and control plots with a trend towards lower numbers in IPM plots late in the season. Results also showed that numbers of natural enemies were higher in IPM plots compared to the control, 30-60% parasitism by *Trichogramma* on striped stem borer and leaf roller eggs were observed in IPM plots in contrast to about 5% in the control, 150-200 kg/ha extra rice was harvested from IPM plots, and pesticide input costs was reduced in IPM plots by about 230 USD/ha (excl. costs for *Trichogramma*) (Hou et al., unpublished data). These results indicate that a well-tuned *Trichogramma*-based IPM based on locally adapted strains of parasitoids, can achieve substantial control of rice insect pests while reducing pesticide use and costs for plant protection, and thus can contribute to the health of agro-ecosystem and sustainable development of rice production. With years of successive practice, a more promising expectation of the beneficial effects of the *Trichogramma*-based IPM can be expected.

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10.3 Conservation Biological Control and IPM Practices in *Brassica* Vegetable Crops in China: A Step Further

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Brassicas (Brassicaceae) are major vegetable crops in China. During the last 30 years, the area of vegetable crops has increased steadily, however, the control of insect pests on brassica vegetables has largely relied on the heavy use of chemical insecticides. Meanwhile, efforts to develop practical and sustainable integrated pest management (IPM) strategies for brassica vegetables have been implemented. Here we introduce one example of *Brassica* IPM practice in the Changjiang river valley of China. Developed in early 2000s, this IPM strategy is based on the cropping system level and the major components include regularly insect monitoring throughout the whole crop season, use a composite and dynamic action threshold for decision-making and strategic use of biological and selective insecticides (Liu *et al.*, 2014). Under this IPM practice, the insecticide input can be reduced by 20-70%, with no risk of of crop loss. However, the apparent effects of natural enemies was usually found in the later crop season probably because of the small farm.

In recent years, the lack of trained farmers constrained the implementation of this IPM practice. The regular sampling and composite action threshold require sufficent specific knowledge on insects. Using sex pheromone trap with automatic-counting device, we explored the possible approach to simplify the insect sampling of key insect pests (Fig. 10.3.1). However, the link between trapped adults and larvae in the plots was disturbed by insect migration.

Fig. 10.3.1. Sex pheromone trap of *Spodoptera litura* with automatic-counting device.

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Besides the reduction of insecticides, providing supplementary resources such as nectar subsidies and habitat manipulation are also practical strategies for natural enemies and for conservation biological control. The important of plants in enhancing the efficiency of natural enemies has been well recognized and "Plant-mediated support system for natural enemies" is developing (Chen *et al.*, 2014). Flower belt of footpath in the field is thought one of the important components for better IPM practices (Fig. 10.3.2).

Fig. 10.3.2. Flower belt of *Astragalus sinicus* in footpath in the field.

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10.4 Biological Control of Olive Fruit Fly in California – Release, Establishment and Impact of *Psyttalia lounsburyi* and *Psyttalia humilis*

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The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), likely originated in sub-Saharan Africa, where the wild olive *Olea europaea cuspidata* L. (Wall. ex G. Don) is found and from which the domesticated olive *O. europaea europaea* L. was derived. Following the path of olive cultivation, *B. oleae* has invaded central and northern Africa, the Mediterranean basin, south-central Asia, and recently California and northwestern Mexico (Nardi *et al.*, 2010). In California, *B. oleae* has spread to all commercial olive growing regions since first being detected in 1998. Researchers have long sought more sustainable management programs for this pest, often by using indigenous natural enemies. In the Mediterranean basin, most indigenous parasitoids found attacking *B. oleae* are generalist ectoparasitoids.

The lack of effective biological control agents attacking *B. oleae* in California led to the initiation of a classical biological control program in 2003. Parasitoids that were imported and evaluated in the University of California, Berkeley quarantine included *Bracon celer* Szépligeti, *Psyttalia humilis* Silvestri, *Psyttalia lounsburyi* (Silvestri), *Psyttalia ponerophaga* (Silvestri), and *Utetes africanus* (Silvestri) (Hymenoptera: Braconidae) (Daane *et al.*, 2015). These parasitoids were reared from *B. oleae* collected from wild olives in Kenya, South Africa, Pakistan, or Namibia (summarized in Daane *et al.*, 2011, 2015; Hoelmer *et al.*, 2011). Also evaluated were the fruit fly parasitoids *Fopius arisanus* (Sonan), *Diachasmimorpha kraussii* Viereck, and *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), each obtained from colonies in Hawaii. *P. humilis* and *P. lounsburyi* have been approved for field release in California (Daane *et al.*, 2015). *Psyttalia ponerophaga* is still under quarantine review. Here, we report on the field release and recovery efforts for *P. lounsburyi* and *P. humilis* (mainly a Namibian strain) in California that were conducted from 2006 to 2013.

Psyttalia lounsburyi and *P. humilis* were supplied by the USDA-ARS European Biological Control Laboratory (EBCL) in Montferrier-sur-Lez, France (2008, 2009, and 2013), the Israel Cohen Institute of Biological Control (ICIBC) in Bet Dagan, Israel (2009–

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2012), and the USDA-APHIS-PPQ, MOSCAMED Parasitoid Rearing Facility at San Miguel Petapa, Guatemala. At all facilities, parasitoids were reared on Medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), cultured on artificial diet. *P. lounsburyi* colonies were established with parasitized *B. oleae* collected from olives in Kenya's Burguret Forest (2002, 2003, and 2005) and Marmanet forest (2007), and South Africa (2005). The *P. humilis* colony was established from *B. oleae* collected in wild olives in Grootfontein and Meteorite, Namibia (2007 and 2008).

Parasitoid releases were conducted in five California coastal counties where the summer and winter temperatures are relative mild. Releases were also made in three inland counties where the summer temperatures are relatively warm (Napa) or hot (Butte and Yolo counties). The release sites were either clusters of ornamental trees, organic commercial olive groves, or abandoned olive groves. Typically, the trees were Manzanillo or Mission cultivars, but some sites had a mixture of cultivars. None of the release sites received insecticides, and the coastal sites were often heavily infested by *B. oleae*, making them ideal habitats for field colonization and establishment of introduced parasitoids.

Pre- and post-release samples were made at all sites. Post-release fruit samples were primarily taken in the spring and fall, when olive fruit fly densities were highest, and began 1–4 weeks after a release, depending on the availability of olives. On each sample date, olive fruit were randomly picked from trees within the release-vicinity, depending on the number of available trees and fruit at each site, resulting in fruit collections that ranged from 102–2,020 fruit per site per sample date. The collected fruit were placed in organdy covered containers, with a raised metal grid on the bottom to facilitate pre-pupal flies dropping from the fruit to be collected and reared to either adult flies or parasitoids.

From 2006 to 2013, we released a total of 40,967 female *P. humilis* and 24,402 female *P. lounsburyi* were released at both coastal and inland sites. Across all sample dates and sites, parasitism by *P. humilis* ranged from 0–25%. Recoveries of *P. humilis* were made immediately following a release date; however, *P. humilis* did not appear to successfully overwinter, and the longest period between a release and recovery date was 193 days. Parasitism by *P. lounsburyi* has ranged from 0-60% per collection. More importantly, recoveries of *P. lounsburyi* were made more than three years after the last release at some sites. Moreover, recoveries of *P. lounsburyi* have been made more than 50 km from any release site.

The field-establishment of imported biological control agents is a major step in a classical biological control program. Releases of North African populations of *P. concolor* have been numerous in Europe; however, these efforts led to *P. concolor* establishment in only southern Italy (Raspi and Loni, 1994) and southern Spain (Miranda *et al.*, 2008). Here, we provide results from the release of sub-Saharan African populations of *P. lounsburyi* and *P. humilis*. We showed recoveries and field dispersal of both *P. humilis* and *P. lounsburyi* within the same fruit season following their releases, and the long-term establishment of *P. lounsburyi*. Many factors could have affected the California establishment of *P. humilis* and *P. lounsburyi*. Foremost was the limited number of parasitoids available to release. In California, maintaining large *B. oleae* colonies throughout the season has been difficult. For this reason, the parasitoids were reared on *C. capitata* in artificial diet, which precluded mass-rearing these parasitoid species in California, where *C. capitata* is a quarantined pest. Tolerance to extreme climatic conditions could be a key attribute influencing the establishment of introduced olive fruit fly parasitoids in California. While both introduced parasitoids were recovered within the same season as the field release, only *P. lounsburyi* appears to have survived the winter. Previous laboratory studies suggest that *P. lounsburyi* is a better match with *B. oleae* regarding thermal performance (Daane *et al.*, 2012; Wang *et al.*, 2012, 2013). Field overwintering survival of both parasitoids was low in California's interior valley, where the summer temperatures are higher and winter temperatures are colder than in coastal olive growing regions, and our results suggest that *P. lounsburyi* survival was higher than *P. humilis* survival due to temperature tolerance (Wang *et al.*, 2013). Continued biological control efforts, therefore, must consider not only parasitoid efficacy based on laboratory trials with an abundance of host material, but on the parasitoid species inherent abilities to survive both climatic extremes as well as periods with low host densities.

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10.5 Biological Control using Predators and Parasitoids in Vietnam: From Successful Participatory Approaches to Potential Challenges

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It has been almost a century since first attempts were initiated in Vietnam with parasitoids *Doryctes strioliger* (Hymenoptera: Braconidae) and *Sclerodermus domesticus* (Hymenoptera, Bethylidae) used against the coffee white stem borer *Xylotrechus quadripes* (Coleoptera, Cerambycidae) (Waterhouse, 1998). In Vietnam 175 parasitoid species [Hymenoptera] of insect pests have been recorded for various crops (Pham, 1996). Microbial agents, notably *Beauvaria* and *Trichoderma,* are commonly used and widely known by Vietnamese farmers. Here we briefly review how the main pests have been targeted by introduction of natural enemies or improved conservation of habitats. We also emphasize the success of participatory approaches, as seen in other Asian countries (Grossrieder *et al.*, 2005).

In coconut, several attempts have been achieved to control the coconut hispine beetle *Brontispa longissima* (Coleoptera: Chrysomelidae). Releases of *Asecodes hispinarum* and *Tetrastichus brontispae* parasitoids (Hymenoptera: Eulophidae) (Fig. 10.5.1) have been made in Central Vietnam (Le and Tran, 2011; Le *et al.*, 2017). More recently earwigs *Chelisoches variegatus* (Dermaptera: Chelisochidae) have been implemented thanks to a participatory training involving farmers (Tran *et al.*, 2013). Rearing methods for earwigs have been set locally, ensuring resilience of local communities and reduced costs.

Fig. 10.5.1. Parasitoid *Tetrastichus brontispae* on *Brontispa longissima* pupae. (Picture: Tran Van Minh).

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In rice, parasitic wasps, hemipterans and spiders are known to exert conservation biological control (Cuong *et al.*, 1997). Field trials using *Trichogramma japonicum* Ashmead (Hymenoptera: Trichogrammatidae) against rice stem borer *Cnaphalocrosis medinalis* (Guenée) (Lepidoptera: Crambidae) were also conducted in North Vietnam (Anonymous, 1977). However, the use of pesticides and the reduced landscape diversity can considerably reduce their survival (Gurr *et al.*, 2012). Using a global approach to tackle climate change issues and reduction of greenhouse gases, Ecological Engineering initiatives using flowering plants *Cosmos sulphureus* Cavanilles (Asteraceae) grown in rice bunds have been developed (Pest Smart) in the province of Bac Lieu (Tra Hat village) (Fig. 10.5.2). Increase of biodiversity and beneficial insects have been showcased in training programs. Conservation biological control (higher parasitism rate) has permitted to reduce brown plant hoppers numbers and pesticides use in treated fields (Sivapragasam *et al.*, 2017). The development of the PGS system (Participatory Guarantee System) has been another example that has reached successful development of promoting the preservation of local natural enemies.

Fig. 10.5.2. Rice bunds flowering plants in Tra Hat, Vietnam.
(Picture: S.L. Khing).

With the rise of new concerns for food security and the fast development of higher food standards (VietGap, Organic Farming, PGS), new natural enemies can be implemented and marketed. Development of natural enemies in greenhouses producing vegetables could be suitable in the highland regions where the highest number of farms are certified Viet Gap or Organic. Public stakeholders can contribute by implementing new policies and foster the development of macrobiological agents.

Despite the benefits of using natural enemies for crop protection and biodiversity, several issues need to be addressed to increase their use. Here we review potential challenges for such development include. One challenge is the traditional system of cropping which consumes high levels of pesticides, and the low costs of pesticides used. Developing participatory approaches to sensitize farmers to exposures to these chemicals, with promotion of agroecological benefits to curb this trend (Escalada *et al.*, 2009). Reduction of pesticide use is one of the first steps to increase conservation biological control. Existing natural enemies can be promoted such as *Neochrysocharis okazakii* Kamijo (Hymenoptera: Eulophidae) against leaf miners *Liriomyza chinensis* (Kato) (Dipetra: Liriomyzidae) (Tran and Ueno, 2012). In several crops, the development of braconid (Sivapragasam and Chua ,1997), trichogrammid (Nguyen and Nguyen, 1982), aphelinid (Dao *et al.*, 2015) or aphidid (Starý and Zelený, 1983) wasps can be considered.

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Session 11: Exploring the Compatibility of Arthropod Biological Control and Pesticides: Models and Data

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11.1 How Differential Stage Susceptibility to Pesticides Affects the Success of Biocontrol Agents

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Pesticides may be more toxic to one life stage of an organism than another. However, many toxicity studies only evaluate susceptibility to one life stage. If differential susceptibility does occur in a population of a species, the question then is what does this mean to populations that we want to protect, such as biological control agents. The implications of differential susceptibility to an insecticide among life stages of the lady beetle, *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae), a common predator of aphids, to population fitness were evaluated using life table data and a population model. The objectives were to determine whether differential susceptibility to a pesticide among various life stages was an important consideration in estimating pesticide compatibility with biocontrols.

Toxicity data for *Coccinella septempunctata* exposed to the neem insecticide, Neemix 4.5 EC was taken from Banken and Stark (1997). In this study, 4th instars were found to be significantly more susceptible to this insecticide than 1st instars (Table 11.1.1).

Table 11.1.1. Lethal mortality estimates by instar for *C. septempunctata* exposed to Neemix.*

Instar	No. tested	Slope ± SE	LC50 (95% CL) (mg/l)
1st	210	1.62 ± 0.46	1,120 (719-3,677)
4th	150	5.22 ± 1.19	520 (405-600)

*From Banken and Stark (1997).

Simulation model: Simulation models based on age-structured Lefkovitch projection matrix models (Lefkovitvh, 1965) were developed to estimate the impact that exposure to neem would have on populations of *C. septempunctata*. Models were developed with RAMAS GIS software. The model consisted of a primary matrix that contained the life-

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history characteristics (survivorship and fecundity) of a *C. septempunctata* population. A starting vector, *n(t)*, of 100 individuals that contained information on the age distribution of the population to be evaluated was multiplied against the primary matrix resulting in a secondary vector, *n*(*t*+*1*), that was then multiplied against the matrix and so on projecting population growth by the time step of the matrix (90 days). The simulation was run with a starting vector [*n(t)*] consisting of the stable age distribution for three time steps.

The acute mortality data for 1st and 4th instars of *C. septempunctata* listed in Table 1 above were used to parameterize the models. We assumed an exposure of 500 mg azadirachtin/l of this insecticide to *C. septempunctata* (Fig. 11.1.1). This exposure level resulted in the mortality levels listed in Table 11.1.2 which were derived by drawing a line from 500 mg/l on the x-axis and reading mortality values from the y-axis. Models were developed with mortality occurring in the 1st instar only, the 4th instar only, and in both instars to compare the results on populations of *C. septempunctata*.

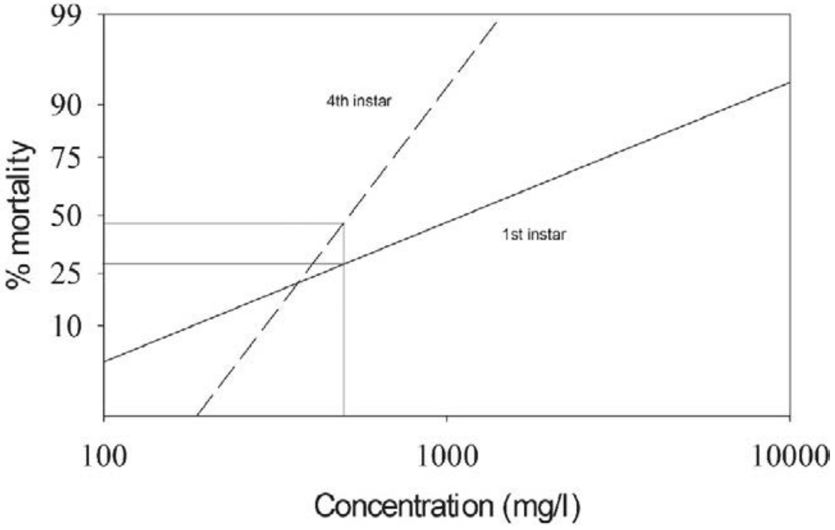

Fig. 11.1.1. Concentration-response curves and predicted mortality of 1st and 4th instars of *C. septempunctata* ladybeetle after exposure to 500 mg/l Neemix.

Table 11.1.2. Predicted mortality of *C. septempunctata* by instar based on the concentration-mortality curves in Fig. 11.1.1 after exposure to 500 mg/l azadirachtin.

Instar	Predicted mortality (95% CL)
1st	29
4th	44

Results of our model simulations showed that a hypothetical exposure of *C. septempunctata* to 500 mg azadirachtin in Neemix 4.5 EC would reduce the number of individuals compared to the control (Fig. 11.1.2). If mortality only occurred in 1st instars, reductions in population size were less than if mortality occurred only in 4th instars. However, the greatest reductions in population size occurred when mortality in both 1st and 4th instars was incorporated in the model (Fig. 11.1.2). In fact, population size in the model where mortality occurred only in 1st instars resulted in a population that was 62% of the control, while the population where mortality only occurred in 4th instars was 43% of the control. The population where both 1st and 4th instars are affected was only 33% of the control.

Results of these model simulations show that differential susceptibility among life stages is an important consideration when trying to estimate the impact of pesticides on populations of biological control agents. If toxicity data are developed for only one life stage, the actual impact that a pesticide might have on a biocontrol agent may be underestimated.

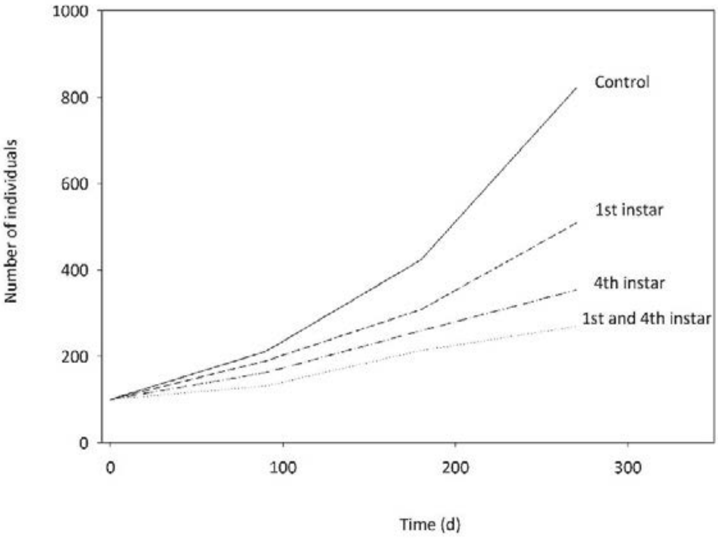

Fig. 11.1.2. Number of individual *C. septempunctata* over time after mortality is imposed on 1st instars, 4th instars, or both after hypothetical exposure to the pesticide Neemix.

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11.2 Orchard Pesticides and Natural Enemies: Lessons from the Lab and Field

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The goal of conservation biological control is to improve existing levels of pest suppression either through habitat manipulation, to attract, retain or improve the performance of natural enemies, or through reduction of pesticide use and selection of products that do not harm resident natural enemies (Heimpel and Mills, 2017). In the latter case, overuse of pesticides, particularly broad-spectrum products, can lead to pest resurgence (outbreaks of the main pest itself) and to secondary pest outbreaks (outbreaks of other pests that live in the same crop) due to release from natural enemy suppression (Dutcher, 2007). Reductions in the frequency or dose of pesticide applications can be effective strategies for conservation biological control, but with the emergence of newer classes of pesticides it is often assumed that reduced-risk products are also less harmful to natural enemies (Gentz *et al.*, 2010). This may not the case however, as reduced risk pesticides often have strong sublethal effects on the behavior and life history performance of natural enemies even when there is less acute mortality (Desneux *et al.*, 2007; Mills *et al.*, 2016).

In apple, pear and walnut orchards in the western USA insecticides are commonly used for control of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), a key pest that is common to these three tree crops. These insecticides can disrupt biological control of secondary pests in these tree crops, such as sap-sucking insects and spider mites (Jones *et al.*, 2009). When considering the selectivity of pesticide products with respect to key natural enemies in western orchard tree crops, several questions arise. Should selectivity testing of pesticides be confined to experimental plots under field conditions; could a more efficient screening of products be achieved under laboratory conditions; and what range of natural enemies need to be tested?

We did a comparative analysis of acute mortality and sublethal effects of orchard insecticides on seven different natural enemy species in a series of laboratory bioassays, and life history responses were measured and integrated into stage-structured population models to estimate population growth rate as a single population-level endpoint. In a parallel field study in which some of the same insecticides were applied in replicated field plots in commercial orchards we examined the hypothesis that laboratory bioassays can be used to rank products for potential impacts on natural enemy populations in the field.

Here I focus on results obtained for natural enemies of the walnut aphid, *Chromaphis juglandicola* (Kaltenbach) (Hemiptera: Aphididae), in walnut orchards, but further details on other natural enemies tested and all three crops can be found in Amaresekare *et al.* (2016), Mills *et al.* (2016) and Shearer *et al.* (2016). The three most important natural enemies of walnut aphid are the exotic parasitoid *Trioxys pallidus* (Haliday) (Hymenoptera: Braconidae) introduced to California as a classic success in importation biological control,

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and two indigenous predators *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae).

From the laboratory bioassays (Table 11.2.1) it is clear that while one of the insecticides tested had uniformly negative effects on all three natural enemies (lambda-cyhalothrin), others had idiosyncratic negative effects on single natural enemy species (e.g., chlorantraniliprole, spinetoram). In addition, some of the observed natural enemy responses were due to acute or chronic mortality (lambda-cyhalothrin) and others were due to reductions in fecundity or egg viability (e.g., chlorantraniliprole and *C. carnea*).

Table 11.2.1. Estimated population growth rates (*r*) for natural enemies of walnut aphid following exposure to insecticides used for control of codling moth in laboratory bioassays.

	Natural enemy species tested		
	Trioxys pallidus	*Chrysoperla carnea*	*Hippodamia convergens*
Control	0.317	0.177	0.112
Chlorantran-iliprole	0.134	-0.107	0.056
Cyantran-iliprole	-0.106	-0.254	0.056
Spinetoram	-0.033	0.02	0.106
Novaluron	0.296	0.042	0.018
Lambda-cyhalothrin	-0.149	-0.174	-0.546

To verify the differential effects of chlorantraniliprole and spinetoram on *T. pallidus* and predators under field conditions, an experimental trial was conducted in a commercial walnut orchard using four replicated 0.5 ha plots for each insecticide treatment. Live aphids, parasitoid mummies and predator larvae were counted every other week from May to October on three leaves from each of five trees in each plot and the insecticides were applied twice (early June and early July) to coincide with the first and second generations of codling moth. Cool weather limited aphid abundance throughout the season and there were not enough predator larvae sampled to support statistical analysis. Although parasitism was significantly lower for spinetoram than the control (ANOVA, F = 6.55, df = 2,9, P = 0.02, Fig. 11.2.1) aphid densities did not differ significantly between treatments (ANOVA, F = 1.79, df = 2,9, P = 0.22).

Three key points arise from this study. Firstly, the laboratory bioassays, which used population-level endpoints informed by sublethal as well as lethal effects (Stark and Banks, 2016), provided a likely ranking of the selectivity of the different insecticides for the same natural enemy species. Secondly, there were considerable differences in the effects of exposure to a particular insecticide between natural enemies in the laboratory bioassays, supporting the suggestion that selectivity likely differs between species as well as between guilds (Banks *et al.*, 2011). Thirdly, location and weather conditions can have an overriding influence on the abundance of secondary pests in large-plot field studies, and this limited our ability to successfully test the relative selectivity of two insecticides on different natural enemy species in a walnut orchard. Nonetheless, spinetoram was predicted to have a greater impact on parasitism than chlorantraniliprole from the laboratory bioassays and this was verified in the field even though aphid abundance was too low to respond significantly to the reduction in parasitism. Thus properly designed laboratory bioassays can be used to screen the selectivity of a broad range of pesticides at low cost, while field studies are better limited to verification of laboratory bioassay results for a limited range of pesticides applied in well replicated trials conducted over several seasons.

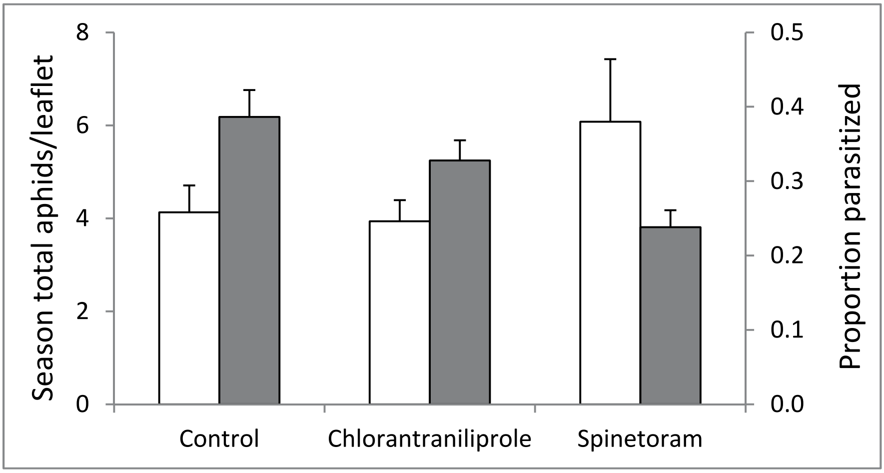

Fig. 11.2.1. Comparative effect of insecticide applications on walnut aphid density (white) and parasitism (grey) in a replicated field trial in a commercial walnut orchard.

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11.3 Pesticide Use and Floral Resources Differentially Affect Communities of Predators, Parasitoids, and Pests in a Regional Survey

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Floral resources may improve the compatibility of biological control and pesticide applications by providing untreated refuge space for natural enemies in crop systems (Lee and Landis, 2001; Roubos *et al.*, 2014), and also by providing non-prey food resources, like pollen and nectar, for omnivorous predators (van Rijn *et al.*, 2016; Gurr *et al.*, 2017). While non-crop plants may buffer negative effects of intense farm management on beneficial insects, these resources may also be exploited by pests and hyperparasitoids (Wäckers *et al.*, 2007), which can compromise biological control. In a two-year survey of brassica crops on more than 50 organic farms in the northwestern United States, we examined farm-scale impacts of both non-crop insectary plantings and use of certified-organic pesticides on the food web associated with aphids, a persistent crucifer pest. We found no evidence that floral provisions buffer beneficial insects against effects of periodic insecticide use in our system. However, floral resources did appear to buffer hyperparasitoids from the negative effects of insecticides, which may indirectly reduce rates of biological control by primary parasitoids. Surprisingly, periodic insecticide use had contrasting effects on generalist predators and primary aphid parasitoids. Aphid densities were also higher on farms that employed insecticides, and the positive effect of insecticide use on specialist parasitoids may be due to the fact that they are tightly linked in time and space with their prey. Altogether, we saw evidence of non-target effects of both insecticides and floral resources that may relax natural pest suppression in our system. Insecticide use was associated with reduced densities of generalist predators and increased densities of aphids, while floral resources reduced the impact of pesticides on hyperparasitoids without significantly increasing densities of parasitoids and predators. Throughout our survey region, farms are small in comparison to larger agronomic regions, and located within a heterogeneous matrix of habit types. When refuge and non-prey food resources are frequently available in natural and semi-natural areas adjacent to farms, floral provisions may have limited benefit (Haenke *et al.*, 2009). Future work should consider both costs and benefits of insectary plantings, as well as the local and landscape variables that determine them.

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11.4 Integration of Biopesticides with Natural Enemies for Control of Tropical Fruit Flies (Diptera: Tephritidae)

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Fruit flies (Diptera: Tephritidae) include some of the most economically important pests attacking soft fruits worldwide including the oriental fruit fly, *Bactrocera dorsalis* (Hendel). The Daniel K. Inouye U. S. Pacific Basin Agricultural Research Center has been a world leader in promoting biological control of *Bactrocera* spp that includes classical, augmentative, conservation, and integrated pest management approaches (Vargas *et al.*, 2012). With the rapid spread of *B. dorsalis* throughout Africa and a related species, carambola fruit fly, *Bactrocera carambolae* Drew & Hancock, to South America, biological control has been a significant component in reducing population levels. In addition, for use against such serious pests as *B. dorsalis*, the Hawaii Fruit Fly Area-Wide Pest Management (AWPM) Program developed and integrated biologically-based pest control technologies that include sanitation, GF-120 Naturalyte Fruit Fly Bait, and lures that are economically viable, environmentally sound and sustainable. The integration of environmentally friendly insecticides with natural enemies was emphasized throughout a 10 year demonstration program (Vargas *et al.*, 2016). Two of the most common *B. dorsalis* natural enemies in Hawaii are *Fopius arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Spatial models and ecological information on these two species was summarized in Vargas *et al.* (2013). In the present study we compared the impact of some commonly used pesticides with biopesticides and cover sprays with bait sprays on these two species.

The efficacies of insecticides against fruit fly adults and parasitoid wasps were compared at the Kona University of Hawaii Experimental Station, Kainaliu, HI. Nine different insecticides (AI/trade name) and or protein bait treatments were evaluated including a water control, Spinosad (GF-120 Naturalyte Fruit Fly Bait), Malathion (Malathion + Nu-Lure Insect Bait (Malathion 5 EC w/ Nu-Lure), Spinosad (Entrust SC), Spinetoram (Radiant SC), Malathion (Malathion 5 EC), Fenpropathrin (Danitol 2.4 EC), Zeta-Cypermethrin (Mustang), Lambda-Cyhalothrin (Warrior II with Zeon Technology), and Tefluthrin (Force CS). Treatments were applied at label rates. Tests were conducted on guava, *Psidium guajava* L. (Myrtaceae) foliage. Twigs with guava leaves were sprayed until dripping, allowed to weather for 1 day, and then exposed for 24 hours to a fixed

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population (20-50 parasitoids) of wasps inside cages (Keiser, 1968; Vargas and Souder 2017).

The effects of the nine insecticides on *B. dorsalis* mortality were reported previously (Vargas and Souder, 2017). Several conclusions were obvious: 1) GF-120 Fruit Fly Bait gave excellent control of *B. dorsalis*, 2) Bait sprays with the same insecticide usually produced higher mortality than with the foliar spray (e.g., GF-120 vs Entrust and Malathion + Nu-Lure vs Malathion), 3) There was considerable variability with foliar sprays; however, many were as effective as current recommended fruit fly treatments, others were not. For example, in initial trials, when compared to GF-120 and Malathion + Nu-Lure, foliar sprays of Warrior II, Mustang, and Malathion gave comparable control; while foliar sprays of Force, Entrust SC, and Radiant SC gave lesser control (Vargas and Souder, 2017). With respect to the male and female mortality of parasitoids to the same nine insecticides (Fig. 11.4.1 and Fig. 11.4.2), two conclusions were apparent. First, lower mortalities occurred with the biopesticides and second, only GF-120 Naturalyte Fruit Fly Bait was effective against the pest *B. dorsalis* and not detrimental to either *F. arisanus* or *D. longicaudatus*. These data support the recommendation of GF-120 Naturalyte Fruit Fly Bait) as part of an integrated pest management approach (sanitation, protein baits and lures) for control of *B. dorsalis* in Hawaii (Vargas *et al.*, 2016).

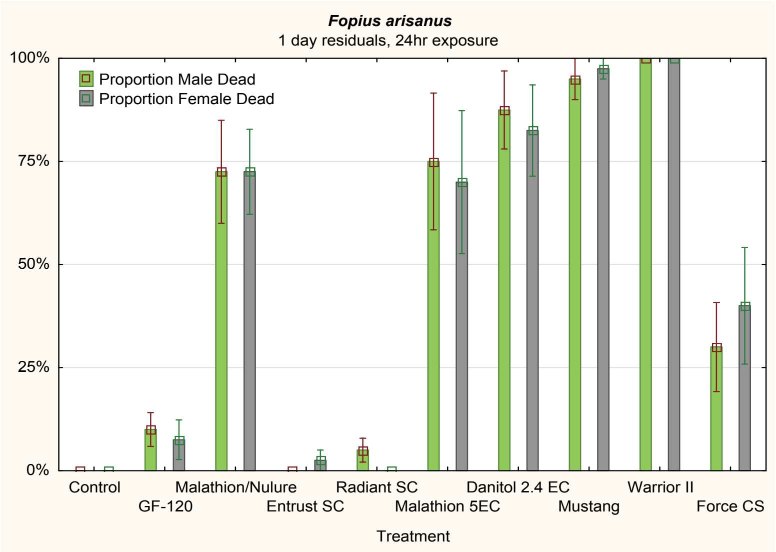

Fig 11.4.1. Mortality of *Fopius arisanus* exposed to nine different insecticide formulations.

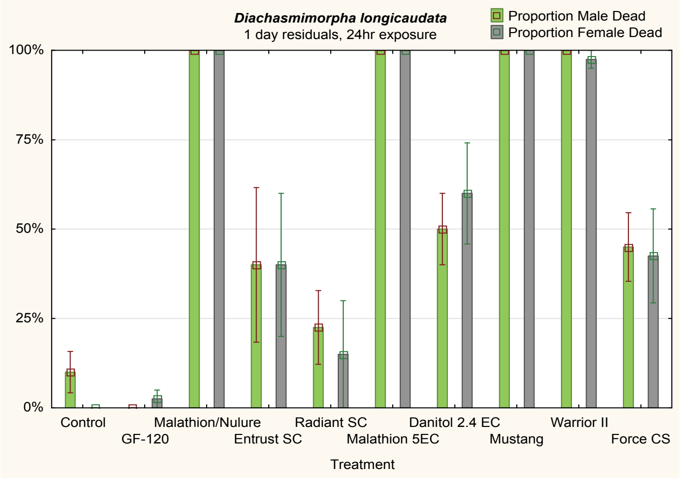

Fig 11.4.2. Mortality of *Diachasmimorpha longicaudata* exposed to nine different insecticide formulations.

Further research is underway to evaluate other insecticides used for control of Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), and development of a systems approach for growers on the U.S. mainland (i.e., California, Texas, and Florida) in response to costly fruit fly quarantines. Findings on the impact of these cover sprays on natural enemies such as *F. arisanus* and *D. longicaudata* will also be included in developing recommendations.

Acknowledgements: This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use. USDA is an equal opportunity provider and employer. We would like to thank the California Citrus Research Board and IR-4 for partial financial support for this work.

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11.5 Protecting Assemblages of Biocontrol Species: Modeling a Surrogate Species Approach

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Parasitoid wasps are important control agents for a suite of economically important arthropod pests, but because of their vulnerability to chemical pesticides they also represent an opportunity to better understand the compatibility of biological and chemical control of pests. Closely related species are often used interchangeably in risk assessment when assessing the risk of pesticides on biocontrol agents. This approach relies heavily on the choice of appropriate indicator or "surrogate" species, an imperfect process often based on phylogenetic or ecology similarity (Murphy *et al.*, 2011; Romeis *et al.*, 2013). We explored the reliability of this surrogate species approach using a population-endpoint perspective. Using a mathematical model parameterized with life history data, we simulated reductions in fecundity and survivorship for a suite of economically important braconid species in order to explore potential differences in their vulnerability to lethal and sublethal effects at the population level.

In the current study, we used a matrix model (Lefkovitch, 1965) to represent the population growth through time of a stage-structured population with four life stages (egg, pupa, larva, and adult). The number of individuals in each of these four stage classes is denoted by X_i for $i = (1,2,3,4)$ with the population expressed as a vector $X = [x_{1,}x_2, x_3, x_4]$. Then the population growth may be described by the matrix equation:

$$X(t+1) = \begin{bmatrix} x_1(t+1) \\ x_2(t+1) \\ x_3(t+1) \\ x_4(t+1) \end{bmatrix} = \begin{bmatrix} 0 & 0 & 0 & f_4 \\ a_1 & 0 & 0 & 0 \\ 0 & a_2 & 0 & 0 \\ 0 & 0 & a_3 & a_4 \end{bmatrix} \begin{bmatrix} x_1(t) \\ x_2(t) \\ x_3(t) \\ x_4(t) \end{bmatrix} = AX(t)$$

where the a_i represent the rate of individuals surviving to the next stage $(0 < a_i < 1, i = 1,2,3; 0 \leq a_4 < 1)$ and $0 \leq a_4 < 1$), and f_4 denotes the reproductive rates of the 4th life stage. In the case of braconid parasitoids, the life stages correspond to egg, pupa, larva, and adult. When the dominant eigenvalue (λ) of the transition matrix (A) is greater than one, then the population will grow (Cushing, 1998; Caswell, 2001). It is possible to relate the dominant eigenvalue to the net reproductive rate of the population, R_0 – that is, the number of offspring produced by one individual during the course of its lifetime. Using this

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approach, we incorporated life table data into the model for four economically important parasitoids: *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), *Psyttalia fletcheri* (Silvestri) (Hymenoptera: Braconidae), *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), and *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae). *D. longicaudata* is used to control a wide variety of fruit orchard pests (Mohamed *et al.*, 2008) and *P. fletcheri* is used to control melon fly *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) (Duan and Messing, 1997). *Fopius arisanus* is an important agent for control of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and Oriental fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) (Vargas *et al.*, 2001); *D. rapae* is an important aphid parasitoid in a wide variety of crops (Costello and Altieri, 1995).

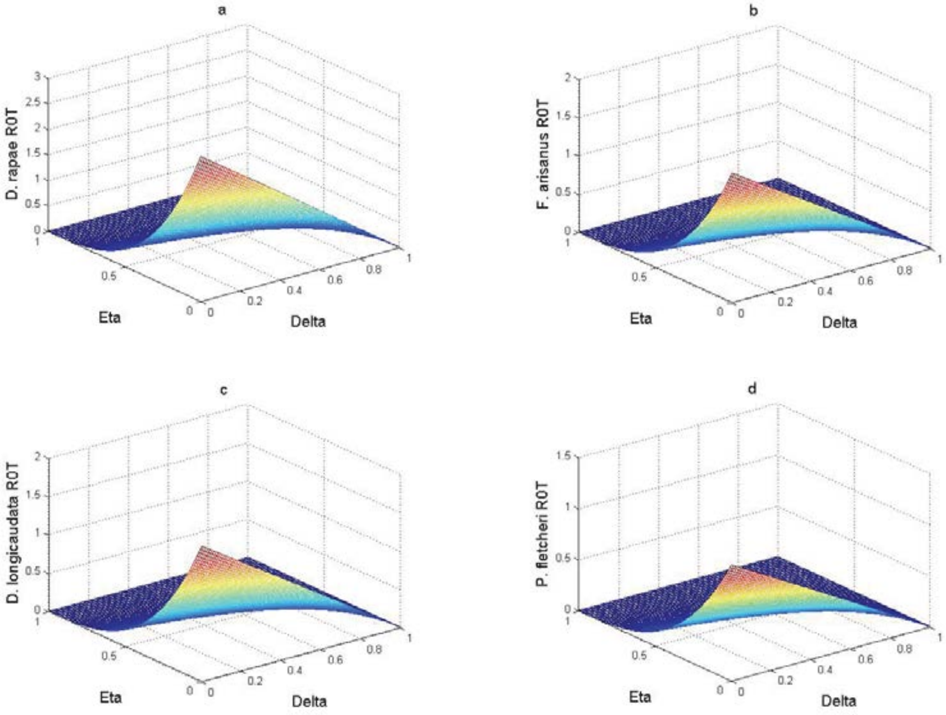

Fig. 11.5.1. Species responses to reductions in survivorship ("Delta") and fecundity ("Eta") for **a)** *D. rapae*, **b)** *F. arisanus*, **c)** *D. longicaudata*, and **d)** *P. fletcheri* (after Banks *et al.*, 2014).

For each of the parasitoid species, we simulated reductions in fecundity and survivorship. *D. rapae* was far more robust than the other three species, persisting even with a nearly 30% reduction in survivorship and almost double that reduction in fecundity (Fig. 11.5.1). The other species responded differently, with *P. fletcheri* being the most vulnerable (Fig. 11.5.1). Our results suggest that the four parasitoid species are poor surrogates for one another. The registration of new pesticides in the United States for the control of terrestrial arthropod pests is based solely on toxicity data stemming from dose–

response testing of the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) (USEPA, 2004). Our findings suggest that using one hymenopteran species to predict the response of another species (even when those species are in the same family) may yield woefully misleading results. We further conducted a sensitivity analysis to determine which life history parameters might be key to best predicting similar population outcomes. Finally, we explored the effects that the evolution of resistance to chemical pesticides might play in the reliability of the surrogate species approach to risk assessment.

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11.6 Disruption of Biological Control Due to Non-target Effects of Pesticides in Australian Grains

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Grains production in Australia covers an area of 22 million hectares, and represents 24% of total agricultural exports. Losses due to invertebrate pests are at least AU$359 million annually, despite pesticides applied as the dominant control option, costing around AU$159 million (Murray *et al.*, 2013; Valenzuela and Hoffmann, 2015; Macfadyen *et al.*, 2016). There are two predominant pesticide application methods for the control of invertebrate pests in Australian grains systems. The first is the application of foliar sprays that are usually broad-spectrum and applied prophylactically, and often repeatedly throughout the growing season. These chemicals typically inhibit enzymes involved in neurotransmission leading to high insect mortality, and can be taken up through direct contact, ingestion and vapour. The second includes more selective chemicals, increasingly applied as seed-dressings (e.g., neonicitinoids such as imidicloprid). Such chemicals are intended to provide protection during initial growth stages of crop plants and may offer both repellent and anti-feeding effects, in addition to pest mortality. The use of seed-dressings often delays or avoids the need for foliar sprays: a shared goal of Integrated Pest Management (IPM).

Using datasets from various research trials conducted in southern Australia, we examined the effects of two different types of pesticides on beneficial invertebrates under field conditions. In the first component of this study we examined the community ecology of invertebrates in a single field over successive seasons under rotation (barley, wheat then canola). Two organophosphates were applied as a control strategy for establishment pests such as earth mites (Acari: Penthaleidae), false wireworms (Coleoptera: Tenebrionidae), earwigs (Dermaptera) and slaters (Isopoda: Armadillidiidae). All ground-dwelling invertebrates were sampled using pitfall and refuge traps. We applied multiple generalised linear modelling techniques using “mvabund” in R (Wang *et al.*, 2012) to understand the changes in pest and beneficial communities. While target pests were suppressed by these treatments, slug (Mollusca) species displayed an increase in numbers in a couple of instances. This secondary pest outbreak appears to coincide with a reduction in the number of predatory beetles that can limit slug populations. High levels of crop damage in the final rotation of canola may be due to slugs being released from predatory pressure.

In the second component we collated datasets from field trials (Jenkins *et al.*, 2013; Macfadyen *et al.*, 2014) that incorporated a randomised block design including a control

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and imidicloprid seed treatments. While regarded as more selective pesticides, the role of neonicitinoids in IPM is debatable as they are applied prior to any economic threshold being reached. Therefore seed-dressings may reduce predator numbers (or perhaps alter behaviour) early in their population growth cycle but not alter risk from pests. Again we used pitfall data and ran multiple generalised linear models on the beneficial communities to look for general trends in response to the different pesticides. As trials were from a range of different parts of Australia, results varied between sites, but there are general trends of the non-target beneficial communities being affected by the application of seed-dressings.

Understanding potential non-target effects of these two chemical control options under field conditions may be used to further IPM approaches. Organophosphates are a cost-effective tool for pest suppression in Australia, although disrupting key predators can reduce effective suppression of other pests, such as slugs, and may lead to secondary outbreaks especially when rotating with susceptible crops such as canola. Selective pesticides administered through seed dressings appear to be softer options, however there is potential to reduce or delay population growth for some beneficial species. Importantly, such effects need to be further assessed in field or semi-field studies to understand the long-term interactions between pesticides, pests, and beneficial communities.

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Session 12: Successes and Uptake of Arthropod Biological Control in Developing Countries

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12.1 Classical Biological Control of Insects in Developed and Developing Countries: a Comparison Using BIOCAT Database

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High-income countries invest more in classical biological control than middle- and low-income countries, and correspondingly benefit more. We analyze the BIOCAT database of insect biological control agents (BCAs) introduced against insects (Cock *et al.*, 2016) to examine the patterns behind this, with a particular focus on low-income countries. The version of BIOCAT used is that of 2017 (Murphy *et al.*, in prep), which includes corrections and missed records, but has not been updated after the end of 2010.

We use the World Bank categories of national per capita income (World Bank, 2017) to classify the countries in the analysis as low-income, lower-middle-income, upper-middle-income and high-income. The term country, which the World Bank uses interchangeably with economy in this context, does not imply political independence but refers to any territory for which authorities report separate social or economic statistics. This classification changes over time, but we have used the most recent classification for our analysis.

Looking at the countries that have at some time made at least one biological control introduction (Table 12.1.1), the low-income countries have as a whole been more active than high-income countries. However, looking at the numbers of countries that have invested moderately (ten or more introductions) or extensively (40 or more introductions), it is clear that the high-income countries have been considerably more active.

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Table 12.1.1. Country uptake of biological control of insects using insects based on World Bank income classification.

World Bank income classification	Number of countries	Number of countries making at least one BCA release	% uptake	Number of countries making at least ten BCA releases	% uptake	Number of countries making at least forty BCA releases	% uptake
Low-income	31	26	84	6	19	0	0
Lower-middle-income	52	39	75	16	31	6	12
Upper-middle-income	56	43	77	26	46	9	16
High-income	79	46	58	25	32	15	19
Total / average	218	154	71	73	33	30	14

Looking at the actual numbers of releases (Table 12.1.2), it is clear that high income countries have made many more introductions than low income countries – 46 high income countries out of 154 active countries have made 66% of all releases, a figure heavily distorted by the USA, which has made 31% of all releases.

Table 12.1.2. The number of biological control introductions of insect biological control agents against insects made by countries based World Bank income classification as at March 2017.

World Bank income classification	Number of countries	Number of countries making at least one BCA release	Total number of releases	Average no of releases (of those making releases)
Low-income	31	26	202	7.77
Lower-middle-income	52	39	712	18.26
Upper-middle-income	56	43	1202	27.95
High-income	79	46	4088	88.87
Total / average	218	154	6204	40.29

The pattern of releases over time (Fig. 12.1.1) has been similar for the high- and middle-income countries, with peaks in the 1930s and 1950s to 1970s (Fig. 12.1.1). However, the pattern in low-income countries differs, starting later and peaking in the 1980s.

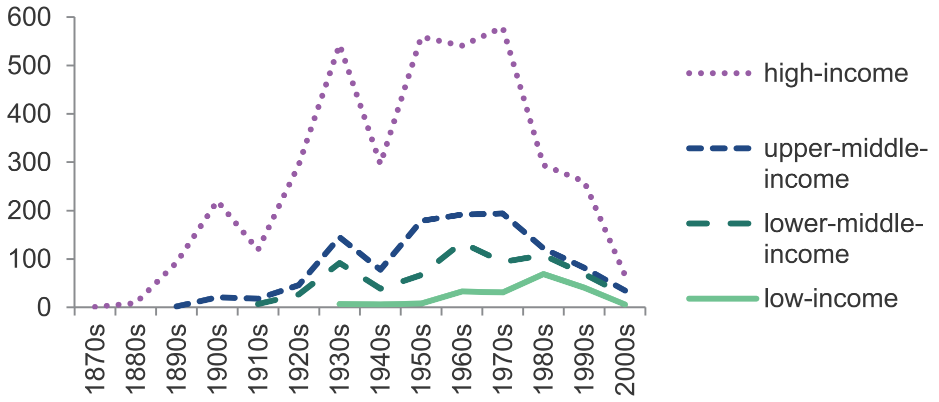

Fig. 12.1.1. Number of insect biological control agents introduced against insect pests per decade, categorised by current World Bank income classification of the target country.

Many classical biological control successes are repeated by using the successful BCAs against the same pests in other countries, sometimes on many occasions. The majority of successful classical biological control programmes in developing countries are of this type (Tables 12.1.3 and 12.1.4). Using known successful BCAs developed by high-income countries is an easy way to tackle some major pest problems in low- and middle-income countries, and this has generated very substantial successes for these countries. However, many important pests in these countries are not a major problem and target for classical biological control in high-income countries and these merit investigation, funded and carried out by the upper-middle-income countries, or funded by donor agencies and carried out by international partnerships.

Table 12.1.3. The number of introductions made of insect biological control agents to control insects in the 31 low-income countries in the current World bank classification.

Number of introductions	Low-income countries
>20	Madagascar, Uganda, Tanzania, Senegal
11-20	Malawi, Comoros
6-10	Togo, Mozambique, Zimbabwe, Benin, Central African Republic, Sierra Leone, Burundi, Democratic Republic of Congo, Guinea
1-5	Rwanda, Gambia, Ethiopia, Guinea-Bissau, Somalia, Afghanistan, Burkina Faso, Eritrea, Haiti, Nepal, Niger
0	Chad, Democratic People's Republic of Korea, Liberia, Mali, South Sudan[1]

[1] Any releases made in South Sudan would appear in BIOCAT2010 under Sudan, as South Sudan did not became independent until 2011.

Table 12.1.4. The number of partial or better successes and the first global classical biological control successes reported from the low-income countries in the current World bank classification.

Low-income country	Number of successes	Globally first successful programmes Target (biological control agent (BCA))	Year
Madagascar	5	African pink stem borer, *Sesamia calamistis* Hampson (BCA: *Pediobius furvus*(Gahan))	1968
Tanzania	5	None	
Zimbabwe	4	None	
Benin	3	Mango mealbug, *Rastrococcus invadens* Williams (BCA: *Gyranusoidea tebygi* Noyes) Larger grain borer, *Prostephanus truncatus* (Horn) (BCA: *Teretrius nigrescens* (Lewis))	1988 1992
Togo	3	Mango mealbug, *Rastrococcus invadens* (BCA: *Anagyrus mangicola* Noyes)	1991
DR Congo	2	Cassava mealybug, *Phenacoccus manihoti* Matile-Ferrro (BCA: *Anagyrus lopezi* (De Santis))	1982
Uganda	2		
Comoros	1	Palm-infesting whitefly, *Aleurotrachelus atratus* Hempel (BCA: *Eretmocerus cocois* Delvare)	2007
Ethiopia	1	None	
Haiti	1	None	
Malawi	1	None	
Niger	1	None	
Rwanda	1	None	

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12.2 Plantwise Data on Extension and the Uptake of Augmentative Biological Control using Arthropods

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Human health issues arising from the use of synthetic pesticides, concerns about their environmental toxicity and issues of pesticide resistance are making biological control agents (BCA) increasingly attractive (Abhilash and Singh, 2009; Chandler *et al.*, 2011). In spite of the large number of available macrobial and microbial augmentative BCAs (van Lenteren 2012; van Lenteren *et al.*, 2017), their market share is still small, representing about 5% of the global plant protection market. There is significant room for growth in the use of BCAs in low- and lower-middle-income countries (as categorized by the World Bank (2017)), particularly in Africa where the uptake of BCAs is still marginal (Olson, 2015).

One of the steps required for the uptake of BCAs by farmers is their recommendation by extension services. Our study focuses on factors that affect the uptake of BCAs for arthropod pests by national extension partners in the agricultural development programme Plantwise. Plantwise is a global programme led by CABI to facilitate the establishment of plant clinics where smallholder farmers can obtain diagnosis and practical plant health advice on written prescription forms (Danielsen and Matsiko, 2016). Extension officers working at plant clinics are supported with offline and online resources which include diagnostic tools, factsheets and pest management decision guides (PMDG, 2017). Using data generated by Plantwise' national extension partners in Ghana, Kenya, Zambia, India, Nepal and Pakistan, BCA recommendations included in nationally produced PMDGs and BCA recommendations given by extension officers and documented in prescription forms were analysed.

Overall, the rate of BCA recommendation in PMDGs for arthropod pests ranged from 13.0% (Zambia) to 61.1% (India). Focussing on the ten arthropod pests most frequently diagnosed at plant clinics in each country, the rate of BCA recommendation in prescription forms ranged from 0.0% (Zambia) to 16.7% (India). It appears that, for most of those pests, BCAs are available in another country, except for major cocoa pests in Ghana. However, they may not be available at a national level, and when they are, they may not be included in the existing PMDGs, they may not be recommended by extension officers, or no PMDGs may be available for the corresponding pest (Table 12.2.1).

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Table 12.2.1. Analysis of the barriers and potential to recommend biological control agents (BCAs) in response to plant clinic enquiries for the ten arthropod pests most frequently diagnosed at plant clinics in each countries: the availability of nationally produced pest management decision guides (PMDGs), the recommendation of BCAs by extension officers, the inclusion of BCA recommendations in nationally produced PMDGs, the availability of a suitable BCA at a national level and the availability of a suitable BCA in another country.

Country	**Percentage ± SE of prescription forms:**				
Number of prescription records for arthropod pests (n=)	**With PMDG available**	**With a BCA recommended by extension officers**	**For which PMDGs have a BCA recommendation**	**For which a suitable BCA is available at a national level**	**For which a suitable BCA is commercially available in another country**
Ghana (2,647)	77.9 ± 2.9	1.2 ± 1.0	10.4 ± 3.9	10.4 ± 3.9	30.9 ± 14.8
Kenya (2,636)	100 ± 0.0	3.4 ± 0.4	43.3 ± 12.5	60.1 ± 5.0	96.3 ± 1.7
Zambia (337)	91.2 ± 4.2	0.0 ± 0.0	17.0 ± 1.9	29.6 ± 3.5	88.3 ± 5.1
India (1,376)	42.2 ± 2.6	16.7 ± 6.9	42.2 ± 2.6	64.2 ± 6.2	100 ± 0.0
Nepal (325)	69.5 ± 10.7	8.9 ± 4.2	18.5 ± 6.9	84.3 ± 10.7	100 ± 0.0
Pakistan (4,100)	68.1 ± 0.5	0.9 ± 0.4	16.9 ± 12.5	37.6 ± 3.7	96.5 ± 3.5

In spite of the large number of PMDGs produced by national extension partners (over 2000 as of December 2016), some major pests are not yet addressed with PMDGs and these gaps should be filled. The relatively low rates of BCA recommendation could in part be attributed to a lack of knowledge among extension workers, and this could be addressed by targeted trainings. Plantwise national extension partners, and extension services in general, should aim to systematically include nationally available BCAs in recommendations given to farmers and in extension material whenever appropriate. Availability of BCAs at a national level is constrained by regulatory frameworks. Registration processes which are not adapted to BCAs have been pointed to as a barrier to the broader uptake of BCAs (Harman *et al.*, 2010; Sundh and Goettel, 2013). Kenya and India have adapted their registration processes to better suit BCAs (Hoeschle-Zeledon *et al.*, 2013), and the larger number of BCAs registered in these countries underline how this affects their availability at a national level. Limited availability at a local level and high prices were identified as further important factors affecting the uptake of BCAs by Plantwise's national extension partners. Better linkages among stakeholders (in particular, manufacturers, retailers, government agencies or trade sector actors) can contribute to the uptake of new technologies (Anandajayasekeram *et al.*, 2008), and such linkages should be encouraged to make BCAs more widely available and to ease their uptake.

The study gives novel insight into the potential of extension services to facilitate the use of BCAs in lower-middle-income countries. By identifying the factors that affect the uptake of BCAs by extension services in those countries, it highlights areas were key players in plant protection can prioritise action, and constitutes a baseline for further studies.

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12.3 The Importance of Local Production to Foster the Uptake of Augmentative Biological Control in Developing Countries

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Augmentative biological control is the periodical release of large numbers of natural enemies mass-reared in biofactories to obtain an immediate control of pests (van Lenteren, 2012). It has been practiced for commercial mass production and sale of natural enemies for roughly 120 years (van Lenteren, 2012) and successfully applied against a range of open-field and greenhouse pests (van Lenteren and Bueno, 2003; Bale *et al.*, 2008). Nevertheless, augmentative biological control is applied on a relatively small acreage (van Lenteren, 2012), even though it has been considered an environmentally and economically sound successful alternative to chemical pest control (van Lenteren and Bueno, 2003). The limited use of augmentative biological control is not only attributed to social and economic factors such as attitudes of the pesticide industry, farmers, governmental institutions and biological control community as well as influence of guidelines and regulations (van Lenteren, 2012), but also due to problems associated with the production and distribution of parasitoids and predators, particularly, the limited shelf life of most natural enemy species (Bale *et al.*, 2008). Some of these limitations may be overcome with evolved government policy to tackle pesticides problems and promote integrated pest management practices, increased demands of food retailers and consumers for safe agro-products and further research. Here we report a grassroot approach towards the establishment of local biological control agent production facilities. Knowledge transfer from an international biocontrol manufacturer to the community level in a developing country was facilitated to overcome the barriers to uptake of augmentative biological control.

Maize, *Zea mays* L. (Poaceae), is one of the most important food crops in the world and, together with rice and wheat, provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries (Shiferaw *et al.*, 2011). In Myanmar, Laos and Yunnan - southwestern China, maize is the most important crop after rice, being used as

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both animal feed and for human consumption. It is grown by approximately 15.4 million smallholder farmers on about 1,871,000 ha, with farm size ranging from 0.2-3 ha (unpublished data). The Asian corn borer, *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae), is the most prevalent and destructive insect pest of maize throughout Asia (Nafus and Schreiner, 1991). In China, annual loss due to this pest ranges from 6 to 9 million tons per year (He *et al.*, 2003). So far, the majority of maize growers in Southeast Asia still use conventional pest control methods, relying heavily on broad-spectrum insecticides. Synthetic insecticides are readily available in China and Myanmar, where fear of crop losses results in overuse. In contrast, farmers in Lao PDR have virtually no access to commercial plant protection products and are thus at high risk of pest outbreaks.

Augmentative biological control with *Trichogramma* (Hymenoptera: Trichogrammatidae) has been used worldwide in a number of crop-pest systems, particularly maize - corn borers (Li, 1994), and has been especially successful to control the Asian corn borer on maize in northeastern China (Wang *et al.*, 2014) and DPR Korea (Zhang *et al.*, 2008, 2010). In recent years, the mass production technology and release technique of *Trichogramma* have been greatly improved, making *Trichogramma* production and field application more practical and cost efficient (Wang *et al.*, 2014). However, smallholder farmers in Myanmar, Laos and Yunnan - southwestern China are unable to fully adopt the approach because they either do not have the knowledge or lack resources and support to access *Trichogramma* products. Therefore, a joint collaboration, funded by DG DEVCO - EuropeAid (DCI-ASIE/2011/261-127), involving agricultural research, development and extension institutions, as well as a commercial biological control manufacturer, was initiated in the three target countries to strengthen intra-regional linkages and transfer of *Trichogramma* production and release technology through south-south partnerships.

Results: Since the beginning of the collaboration, a grassroots approach was employed to facilitate the active participation of all relevant stakeholders and strengthen the links between them, particularly smallholder farmers being brought into the mainstream of technology transfer and playing a key role in the decision-making process. Involving multi-institutional representatives from the target regions, an intra-regional knowledge and exchange platform was established to strengthen south-south partnership and to facilitate the transfer and knowledge and the *Trichogramma* production and release technology. A Chinese production technology for *Trichogramma* was adapted to the hotter climates and the specific needs of management by small-holder maize farmers, and as release strategies.

In total, eight smallholder communities (3 in Myanmar, 4 in Yunnan - China, 1 in Laos) covering approx. 8000 smallholder maize farmers were mobilized and established local grassroot organisations responsible for i) training of facility personnel to produce *Trichogramma* and farmers to apply the *Trichogramma*, ii) management of the *Trichogramma* production facility, according to a business plan, and iii) marketing of the *Trichogramma* and improved access to market opportunities. Such an innovative community-based approach is instrumental for long-term sustainability of the established *Trichogramma* production facilities, ensuring that profits stay within the community and inheriting the promotion of product and the ecologically-friendly farming practices at the community level.

About 37 rearing technicians were trained on *Trichogramma* production and over 5000 farmers were trained on *Trichogramma* application through participatory-based training of trainer approach. Through stepwise approach, eight *Trichogramma* production facilities have been established and produced *Trichogramma* egg cards in standard quality but still have a way to go in order to reach a higher production capacity and thus the economic break-even point. The facilities were providing not only *Trichogramma* products but also other essential agricultural inputs and marketing services as well as associated capacity building to catalyse the uptake of the integrated pest management practices by smallholder maize farmers. Moreover, national or regional stakeholder conferences and local awareness/promotion days were organised in three target countries to improve awareness and create conducive environment for large-scale dissemination of the available biological control technology for the first time in the region. Over 9000 participants were attracted to these awareness creation events, including local farmers, extension staff and officials from local and regional governments.

Discussion: Many farmers in the developing countries face several barriers to adopting new and potentially profitable agronomic practices and technology, during which access to information, input and output markets and services play a critical role (Shiferaw *et al.*, 2011). Deployment of biological control agent production facilities at the community level would facilitate farmers' access to a tangible biological control product and promote adoption of augmentative biological control technology in developing countries. In our case, farmers are not passively receiving a technology but rather proactively involved in technology adaptation and adoption. Farmers also learnt how to operate and manage the facilities in a business way. However, future success is largely dependent on a great level of investment by public and private sector and sustained political commitment from developing country governments to upscale these innovations through enhanced support for extension and marketing services and increased inputs and value chain development.

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12.4 Understanding the Ecology and Impact of Parasitoids of the Whitefly (*Bemisia tabaci* Complex: Aleyrodidae) in Cassava Landscapes of East Africa

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Over the last 20 years there has been an increase in outbreaks of the whitefly pest complex, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), in cassava production landscapes in East Africa. This species complex transmits plant viruses that have caused widespread damage to cassava, a staple food in many households. In addition to plant disease symptoms, *Bemisia tabaci* populations, when they reach very high numbers, cause damage from direct feeding and excreting sugar-rich exudate; this encourages sooty mold on the leaf surface, reduces photosynthesis and causes further yield losses. Whilst significant effort has gone into developing virus-resistant cultivars, less attention has been paid to the management of natural enemies of *B. tabaci* that may provide biocontrol services. Furthermore, the role that naturally occurring parasitoids play in preventing or dampening outbreaks is unclear (Fig. 12.4.1).

A comprehensive picture of the common parasitoid species that cause significant mortality to *B. tabaci* species found on cassava is not available. This is partly due to recent (and ongoing) molecular clarification of the phylogeny of the *B. tabaci* species complex (De Barro *et al.,* 2011) and unexplored parasitoid species diversity in East Africa. A list of the parasitoids attacking the *Bemisia* genus in Sub-Saharan Africa included eight or more species, with *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) and *Encarsia sophia* Girault (Hymenoptera: Aphelinidae) and Dodd being the most often recorded (Legg *et al.*, 2003). More recent surveys of *B. tabaci* parasitoids in cassava in Tanzania found 10 species of parasitoids (Guastella *et al.*, 2015). Given that there are likely to be more parasitoid species present than currently recognized, we urgently need to use both morphological and molecular techniques to identify the parasitoid species diversity in this region and to determine the most numerically common species in order to focus future research on harnessing their naturally occurring biocontrol services.

Hoelmer (1995) summarized several papers that suggested that parasitoids may be insufficient to control *B. tabaci* without other control methods. Similarly, in cotton systems in America, predation was found to be the key factor determining intergenerational

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variation in mortality. Parasitism was considered the factor contributing least to marginal mortality of immature *B. tabaci* on cotton (other factors such as dislodgement were greater) (Naranjo and Ellsworth, 2009). However, in other systems parasitoids do play an important role. *Eretmocerus hayati* was introduced into Australia and in combination with the careful use of pesticides has improved the level of control of *B. tabaci* Middle East-Asia Minor 1 (MEAM1, formerly called the B biotype) in vegetable systems (Villanueva-Jimenez *et al.*, 2012). Furthermore, there are management strategies that could be used to increase the abundance or activity of parasitoids that have not yet been explored for East African cassava production landscapes.

Fig. 12.4.1. Parasitized nymphs of *Bemisia tabaci* on the underside of a cassava leaf, which have a black colouration. Two non-parasitized *B. tabaci* adults can also be seen in the process of emerging. (Photo credit: CSIRO).

A small amount of research has been completed to quantify the impact of parasitoids on *B. tabaci* on cassava. In Uganda, *Eretmocerus mundus* and *Encarsia sophia* were responsible for 34% parasitism of fourth instar nymphs (Legg, 1995). Percent parasitism was recorded as <20%, and on three occasions <50%. However, there was a negative relationship between parasitism rate and nymph numbers indicating that these parasitoids may not respond in a density dependent manner (Otim *et al.*, 2006). Life history studies conducted under field conditions showed that parasitism caused the highest mortality to fourth instar nymphs, but dislodgement was the key mortality factor for eggs (Asiimwe *et al.*, 2007). Survey data from four regions in Uganda showed that parasitism rate on field-collected nymphs was 40-58% (Otim *et al.*, 2005).

There has been little research to understand how different cassava cultivars might influence the activities of natural enemies of *B. tabaci*. Across East Africa there is a large diversity of local cultivars, including those that have been bred to possess a range of tolerance or resistance to viruses. However, a comprehensive understanding of how the traits of each cultivar impacts higher trophic levels is unknown. Otim *et al.* (2008)

conducted some research to look for cultivar effects on parasitoids, using a comparison between a glabrous and hirsute cultivar. Leaf hairiness in one cultivar caused some changes in parasitoid behaviour, but did not reduce parasitism.

Given the long growing season of cassava and the low level of pesticide use by East African smallholder farmers, the use of parasitoids in combination with other approaches to control *B. tabaci* in cassava is worth exploring further. We conducted a broad survey of the spatial patterns of activity of parasitoids from cassava growing regions in Uganda, Tanzania and Malawi. We discuss the information needed to assess impact in terms of potential for biocontrol of *B. tabaci*. Developing biocontrol options for farmers involving both predators and parasitoids will be important elements of future management strategies that address the *B. tabaci* problem in East Africa.

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12.5 Success and Failures of IPM in Africa and Asia: the Significance of Biocontrol

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Integrated Pest Management (IPM) is defined as farmer driven, ecologically based pest management that has the aim of reducing reliance on synthetic chemical pesticides. Globally, the technology has received much research and promotion effort since the early 1970s and is still regarded by many as the optimum pest management strategy (FAO, 2017). Nonetheless, adoption of IPM in developing countries remains low, despite some successes against particular pests, and efforts to promote its use via farmer participatory approaches. Several reviews to assess adoption of IPM were made in the early 2000s (Maredia *et al.*, 2003; van den Berg, 2004) that subsequently led to many studies on successes and constraints to adoption. However this effort has been scattered, with divergent methods and views often expressed. A recent review of obstacles to the adoption of IPM in developing countries focused on the analysis of responses from IPM professionals and practitioners from 96 countries (Parsa *et al.*, 2014). The main obstacle identified by IPM professionals was "insufficient training and technical support to farmers", although these views differed between key informants from developed and developing countries.

In this paper, we report on an on-going meta-analysis of published literature on the CAB Direct (2017) platform that was undertaken to identify validated evidence of sustainable smallholder IPM implementation. The paper focuses on the uptake of IPM programmes in Africa and Asia, the factors contributing to its successes and failures, as well as the particular role of biological control (biocontrol) activities in these programmes.

Methods: Two main searches were undertaken in CAB Direct: "IPM SUCCESS" / "IPM FAILURE", and "IPM and EVALUATE and AFRICA" / "IPM and EVALUATE and ASIA". Papers were excluded if they fell outside of geographical scope; were general overviews; or if they reported results from experimental trial. Papers were categorised into: geographic regions/countries; date of research/paper; primary research IPM outcome or review; cropping systems covered; and pests covered. The final papers selected were analysed for successful and failed implementation and uptake of IPM, and the significance of biocontrol in outcomes. Authors definitions of "success" and failure" were used in the analyses. This analysis was conducted using a mixed method approach.

Results: The CAB Direct searches returned 460 papers. The IPM success search returned 350 papers: 223 were excluded as they fell outside of geographical scope and a further 97 were excluded as they were experimental or not relevant for other reasons. This left 29

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papers for the IPM successes. Of the 110 papers found in the IPM failure search, 96 fell outside of geographical range, and 11 were on experimental work so were excluded. This left 3 papers for IPM failures. Some of the papers identified under IPM success gave results as ‘mixed’. The search on IPM evaluations in both continents mostly highlighted papers that were found in the first search.

In total, 32 papers were used for further analysis; of these, 29 report a ‘moderate to total’ success. Ten papers covering fourteen projects were the results of direct implementation outcomes, whilst the 22 others were reviews of other work. The ten papers were used for the main analysis of outcomes, but additional material was taken from the review papers if it yielded any new information.

In general, the majority of the papers dealt with pests on rice and cotton in Asia – particularly Indonesia and India (Fig. 12.5.1). The wide coverage and implementation of the FAO IPM initiative from the late 1980’s to the early 2000’s accounted for many of the records from Indonesia. Records relating to work in Africa in the primary search mostly covered attempts to implement IPM based on the successes of IPM schemes in Asia. There was also a lack of reporting of failures (or using the word ‘failure’) in IPM.

Factors used to measure the outcome of IPM implementation: The most common measures used to evaluate measure of successes or failures of IPM implementation are changes in crop yields and profit to farmers. Other measures include: changes in pesticide usage/frequency of spraying, extent of effort to implement IPM adoption, and farmer knowledge of natural enemies. However, factors that affect the outcomes of IPM adoption are different to measures of success. A summary of the main factors identified in the primary research and review papers influencing the outcome of IPM are listed in Table 12.5.1. In Africa in particular, inappropriate agricultural policies, lack of market information and poor infrastructure hindered the success of IPM (Nwilene *et al.*, 2008).

Fig. 12.5.1. Map showing the locations and numbers of papers from the success and failures IPM lists.

Table 12.5.1. Summary of main factors in the primary research and review papers influencing the outcome of IPM.

Factor affecting outcome	Constraints mentioned in the papers
Legislative and policy support	Conflicts between national organisations reduce efficacy and support of interventions
Adoption leads to higher yields	Main factor that make farmers accept or reject concept of IPM
Farmer knowledge	Farmer field school teaching tools are important; knowledge does not diffuse through to untrained farmers
Complexity of information	Pest or economic thresholds too complex for uptake and diffusion; lack of targeted information on IPM techniques hinders farmers with poor education
Continuity in extension	Regular follow up sessions are crucial to deal with changing conditions; extension staff have too many responsibilities
Land ownership	Lack of land ownership means farmers are not as motivated to improve soil health
Age of farmers	Farmers under the age of 40 were more likely to adopt IPM practices than those over the age of 40

The significance of biocontrol in outcomes: Biocontrol was a key practice in all but one of the 14 primary research projects (covered in the 10 primary research papers). The projects principally involved the conservation of native natural enemies, and were mainly focused on cotton and rice systems. All but one was deemed a success (with a few mixed results). The success of a biocontrol activity, usually highlighted as the principal component of the IPM system, is most commonly assessed as diversity of natural enemies and/or actual impact on pests in the crop system. However, the sustainability of a successful biocontrol IPM system is a controversial subject, as some authors have questioned whether the approach can be sustained in the longer term (e.g., Nwilene *et al.*, 2008). Hence this research analysed the main factors identified as being important to longer term biocontrol success in Table 12.5.2.

Table 12.5.2. Main factors mentioned in primary research papers affecting biocontrol in IPM.

Factor affecting outcome	Constraints mentioned in the papers
Farmer knowledge	Lack of knowledge, particularly about natural enemies and beneficial insects is the principal factor affecting uptake of biocontrol. Complexity of language also makes it hard for farmers to relate to technologies
Farmer community cohesion	Lack of community cohesion to develop area wide solutions to deal with pests and diseases. Knowledge and solutions spread more easily in places where farmers work more as a community
Fear of failure in crop production	Farmers who use biocontrol in IPM still resort to the most toxic pesticides in the first instance if they fear they will lose their crop

The subsequent analysis of the IPM reviews observed the messaging used to transfer knowledge to farmers was a vital component of a successful IPM implementation (e.g., the use of insect 'zoos). Farmers have found other concepts in IPM difficult to understand and/or use.

Conclusions: The CAB Direct search, and subsequent primary research analysis, yielded three times more IPM success documents than failures. Various theories can explain this: the terminology used by authors to describe their work was different, and was not picked up by CAB Direct. People are also far more likely to document their successes than their failures. Finally, professionals with a pro-IPM background might concentrate on the success of the intervention rather that its failures in order to drive a certain agenda. However the authors believe that failures are as important to document as successes in order to learn social, economic and ecological lessons from the field. This ensures mistakes are not repeated. The review could also be improved by an added search of other academic databases, like the Web of Knowledge, Web of Science and Google Scholar.

This paper also points towards the lack of primary and implementation research being conducted in Africa and Asia, compared to the wealth of research in Europe and North America. Whilst 19 Africa specific papers were returned, these were mainly due to the large IPM programmes (i.e. FAO, UN development programme, etc.) instigated in the last 20 years. This indicates there is a lack of published knowledge on the outcomes of these schemes, as well as a lack of impetus from national and local research organisations.

Policy is the most significant factor affecting IPM uptake success or failure in Africa and Asia: the judicious use of incentives and reduced subsidies for chemical control are good examples of successful policy implementation for the adoption of IPM (Resosudarmo, 2012). However, the importance of individual farmers' ability to understand and undertake IPM specific activities should not be underestimated. Education and communication, as well as the strengthening of community processes and local rural advisory services' support are vital for the long term success of IPM in the field.

Finally, this review demonstrates that biocontrol is an essential part of IPM, and adds value to any management strategy if developed correctly and in a participatory fashion. This analysis, and indeed any further IPM implementation projects, would be strengthened by an improved understanding of the economics of biocontrol, as estimated by Naranjo et al. in 17 different projects in 10 countries (Naranjo *et al.*, 2015). Whilst the domain is fraught with unknowns, such as the difficulty of estimating all costs, especially environmental ones, it is possible to make advances by encouraging scientists to think beyond the traditional borders of their field and collaborate to form more holistic cost benefit analyses.

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Session 13: Socio-economic Impacts of Biological Control

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13.1 Success and Impact in Classical Biological Control: Some Examples from Developing Countries

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Classical biological control (CBC) is the introduction of a biological control agent (BCA), usually from a pest's area of origin, to permanently control a target pest in an area where it has become invasive. In the context of agriculture, the main beneficiaries of CBC are the farmers who have their pest problems reduced without necessarily actively promoting or introducing BCAs, which by spreading and reproducing naturally contribute to the public good (Cock *et al.*, 2010). The reduced crop losses from pests lead to improved food security and improved livelihoods. Farmers in most parts of the world have benefited from this approach. Farmers also benefit from reduced exposure to pesticides as a result of reduced need, and consumers benefit from reduced pesticide residues in food. Thus, CBC is a public good, as the benefits reach all who grow and use the crop, without requiring them to make any additional interventions. The use of CBC also enables producers to reduce pesticide use and residues to meet the high standards of profitable foreign export markets, resulting in job creation in the grower's country and a significant influx of foreign exchange to developing countries. For all these reasons, CBC in developing countries is often considered an appropriate form of development assistance, and suitable for development agency funding.

In this analysis, following Cock *et al.* (2016), we shall use the terms 'success' and 'successful' to refer to the extent to which a CBC programme has been carried out as planned and the target pest brought under control, and the term 'impact' to assess how this success has addressed socio-economic and societal concerns (normally positively, but the possibility of adverse effects should not be ignored).

Thus, when evaluating the success and impact of a CBC programme, after the initial selection of the target, factors to consider include:

- The logistics of implementation: was a suitable BCA found? Was it successfully released in the target area? Did it become established and persist in the target area? When starting a new CBC programme, there is no guarantee that any of these steps can be completed, and their achievement needs to be documented.
- Is there any evidence to show what impact the BCA has had on the target pest? Can it be demonstrated that the BCA successfully reduced the population of the target pest?
- Has reducing the population of the target pest led to the desired benefits in terms of reduced pesticide use, reduced crop losses, increased carrying capacity, increased yields and/or environmental benefits?

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- What economic, ecological and social changes have these benefits produced, and do these align with the goal of the intervention? In recent years, donors increasingly want to see a proper gender-sensitive impact evaluation, to help show how their funding has contributed towards achieving their objectives such as the Millennium Development Goals.

It may be helpful to consider these steps in terms of the logical framework approach, often used for monitoring the implementation and impact of development projects (NORAD, 1999). The steps in a CBC programme are the activities, which if successfully implemented lead to outputs and an outcome, the biological control of the target pest, i.e., a success. Beyond this relatively straightforward success, the project purpose will be achieved if the successful biological control leads to increased yields or reduced losses in the crop system (this will be different for environmental pests), and if so whether this contributes to the overall goal, which may be characterized as improved or protected livelihoods, moving people out of poverty, etc.

We consider five case studies (Table 13.1.1). The types of negative impacts caused by the pests include:

- Crop losses due to direct damage by the pest
- Increased pest management costs and time inputs, e.g., pest monitoring, extra pesticide applications, additional cultural control measures, weeding
- Increased pesticide use and associated human and environmental harm
- Production of certain crops may cease to be viable if all or most of the crop is lost
- Reduced, poor quality, pest contaminated or pesticide contaminated export crops leading to consignments being turned away, affecting national economics
- Market prices rise due to crop shortages, adversely affecting quality of rural / urban nutrition
- Loss of ecosystem services such as watershed protection, pollination, etc.

There are also knock-on effects of these negative impacts, including:

- Livelihoods of rural subsistence farmers, through reduced food available for on-farm consumption, reduced family income from selling produce, increased crop production costs
- Increased food prices if the amount of locally produced food goes down
- Negative impact on food security due to reduced staple food production
- Negative effects on the national economy due to changes in local food markets and export crop earnings

Table 13.1.1. Overview of the impact assessment of five case studies of successful classical biological control of insects using insects.

Common name	**Target area**	**Key pest impacts**	**Type of published impact assessment**	**Cost: benefit (region)**	**Scope for further evaluation**
Cassava mealybug *Phenacoccus manihoti* Matile-Ferrero (Hemiptera, Pseudococcidae)	Tropical Africa	Cassava crop losses threatening livelihoods and food security	Detailed scientific assessment and cost-benefit analysis	94 – 800 (Africa)	The programme in Africa has been documented, but the programme in SE Asia is on-going.
Mango mealybug *Rastrococcus invadens* Williams (Hemiptera, Pseudococcidae)	West Africa	Mango and other fruit crop losses threatening livelihoods and quality of nutrition	Detailed scientific assessment and cost-benefit analysis	145 (Benin)	The programme in Africa has been documented, particularly in Bénin, but there is scope for extrapolating impact across the region.
Pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) (Hemiptera, Pseudococcidae)	Caribbean	Multiple crop losses affecting livelihoods and trade	Some scientific assessment and partial retrospective cost-benefit analysis	8 (Trinidad & Tobago)	It should be possible to make a more detailed Caribbean-wide cost-benefit assessment.
Brown peach aphid *Pterochloroides persicae* (Cholodkovsky) (Hemiptera, Aphididae)	Yemen	Peach and stone fruit crop losses threatening livelihoods	Limited scientific assessment and anecdotal comments on socio-economic impact	–	Not practical at present, given the fighting in Yemen. In the future, monitoring of aphid and BCA would be needed to evaluate success; data on the value and importance of stone fruits for livelihoods will be needed.
Banana skipper *Erionota torus* Evans (Lepidoptera, Hesperiidae)	Mauritius	Banana losses affecting industry and quality of nutrition	Anecdotal comments on control of target and benefits used to extrapolate based on economic figures	53 (Mauritius)	Monitoring of skipper and BCAs is needed to clarify if CBC is working effectively. A more rigorous cost-benefit analysis should be made.

Discussion: The final impact envisaged in the planning and preparation stage of a CBC project will have implications. What impact is desired and why is it thought that the successful biological control of one particular pest will produce this impact? In some cases, the argument for tackling a new alien pest is very clear-cut, but this is not always the case, especially when ecosystem interactions are more complex than most agricultural ecosystems, e.g., many weeds and environmental pests. Most such ex ante studies have focussed on the anticipated costs of pests based on yield losses and control costs, but Hill and Greathead (2000) extend this to include the area at risk of infestation, damage level, indirect damage (e.g., side effects of pesticides or loss of habitat) and amenity costs (quality of life, human health, environment, social and cultural practices). A clear theory of change may be needed to pinpoint what impact is intended, and what impact is desirable but not the primary objective.

As our case studies and many other publications have shown, there have been many substantial successes using CBC. However, impact is often poorly assessed, if at all, and is seldom documented, especially in developing countries. There is some limited scope for retroactive studies, structured around a cost-benefit analysis, but in most cases rigorous evaluation of the socio-economic impact is no longer practical. Commonly used socio-economic methods can be and have been adapted to assessments of BCA impacts and tested to see if the tools are appropriate. It is likely that the tools would need to vary slightly to assess impact of an agricultural pest as opposed to an environmental pest. Protocols could most easily be developed and tested in programmes that reuse BCAs of known effectiveness, where basic parameters are reasonably well understood. Fine tuning some protocols specifically for these impact areas could be useful as it would provide some examples of standard good practice to work from in future programmes, especially for those who do not have a socio-economic background.

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13.2 Cost of Biological Control of Invasive Arthropods

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California is the largest producer of perennial tree and vine crops in the U.S. In 2015 over 12.4 million utilized tons were produced on 2.5 million acres for a total value of over $16 billion (USDA-NASS, 2017). California agriculture has also seen the establishment of invasive arthropods that affect the costs to produce fruit. These pests can be treated with conventional or biological pest controls.

Between 2000 and 2015 acreage treated in both conventional and biopesticides for arthropods increased. However, while there was an increase in conventional insecticides, total acreage treated in biopesticides in 2000 was greater than the total acreage in 2015 (Figs. 13.2.1 and 13.2.2) (CDPR, 2017). Increased treatments are a result of increased acreage in production, and the entry and establishment of invasive arthropods during this time period such as the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), and olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae).

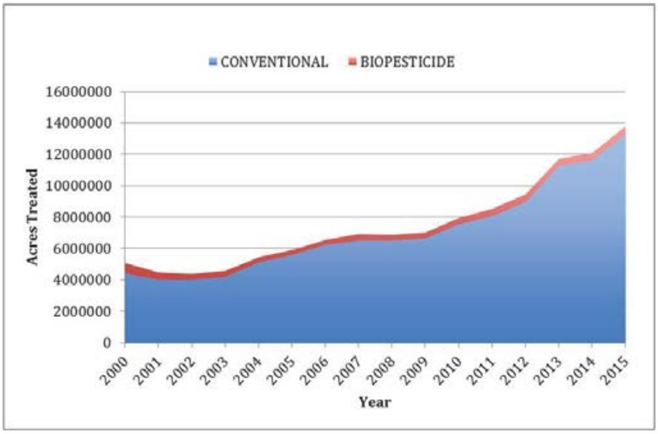

Fig. 13.2.1 Acreage treated in conventional and biopesticides.

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While overall acreage treated in biopesticides was lower in 2015 than in 2000, the mix of biopesticides has changed and there has been an increase in the use of phermones and "other". Other biopesticides include insect growth regulators such as azadirachtin, and microbial insecticides such as Grandevo.

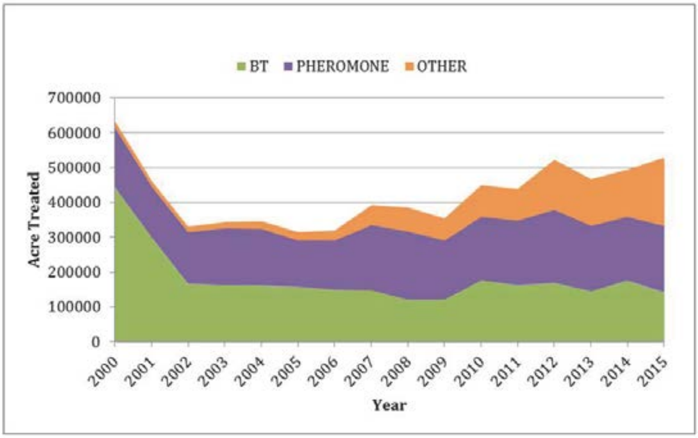

Fig. 13.2.2. Acreage treated in biopesticides by category.

In recent years acreage increasing in biopesticides has been increasing more rapidly than acreage in conventional pesticides, also causing costs to increase. For example, between the years 2011 and 2012 acreage treated with conventional insecticides increased by 9.0% while costs increased by 10%. During that same period acreage in biopesticides increased by 36% while costs increased by 39% (Table 13.2.1). However, the increase between 2012 and 2013 was even greater. Acreage in conventional insecticides increased by 26% while costs increased by 17%. Acreage in biopesticides increased by 155% while costs only went up by 135%.

Table 13.2.1. Acreage treated and cost of treatment for conventional and biopesticides.

	Acreage		Costs (US $)	
	Conventional	**Biopesticides**	**Conventional**	**Biopesticides**
2011	4,134,371	2,729	354,282,036	166,207
2012	4,579,277	3,808	386,195,689	226,368
2013	5,397,497	8,979	488,531,205	487,647

In addition to biopesticides, classical biological control programs have also been completed and agents released into the environment. These agents can cause significant cost savings depending on crop and pest type.

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13.3 Assessment of the Economic and Poverty Impacts of Biological Control of Cereal Stemborers in Kenya using the Economic Surplus Modelling Approach

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In response to the severe stemborers invasion in cereal farming regions of Kenya, a biological control (BC) program was initiated by icipe scientists. This program has released four natural enemies: *Cotesia flavipes* (Cameron), *C. sesamiae* (Cameron) (Hymenoptera: Braconidae), *Telenomus isis* (Polaszek) (Hymenoptera: Scelionidae) and *Xanthopimpla stemmator* (Thunberg) (Hymenoptera: Ichneumonidae) to suppress economically important stemborer pests; *Busseola fusca* (Fuller), *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Chilo partelus* (Swinhoe) (Lepidoptera: Crambidae) . An economic surplus model was developed, based on time-series data on production, market and GIS, to evaluate the economic impact of the BC program in Kenya. Findings show that the BC intervention contributed to an aggregate monetary surplus of $US 0.74 billion to the economy of Kenya. The net present benefit of $US 141.52 million, the Internal Rate of Return of 113.08% and the Benefit Cost Ratio of 276:1 justify that the program was worthwhile. An estimated average of 57,400 persons were yearly lifted out of poverty due to the BC program. Wide promotion and up-scaling of the BC program should thus be considered.

Background and Objectives: In Eastern and Southern Africa, cereals, especially maize, *Zea mays* L., and sorghum, *Sorghum bicolor* (L.) Moench (Poacaea), are among the most important and widely grown field crops but their production is constrained by biotic and abiotic problems. Among biotic constraints, insect pests represent an important challenge and stemborers are by far the major injurious pests in maize and sorghum production (Omwega *et al.*, 2006). Biological control (BC) or use of natural enemies of the pests offers an alternative economically, socially, and environmentally friendly strategy to the frequently used expensive and harmful pesticides (Naranjo *et al.*, 2015). The International Centre of Insect Physiology and Ecology (icipe) undertook a BC program that involved mass-rearing and release of natural enemies to regulate stemborers pest populations. While the acceptable level of parasitism, stemborer densities and yield loss effects are well documented (Omwega *et al.*, 2006 and references therein), economic benefits and poverty reduction impacts of the BC program has not been established. This study analyzed the

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welfare effects of the release of natural enemies of maize and sorghum stemborers through different BC programs initiated by icipe from the early 1990s to 2007, and the return to investments in research on icipe implemented biological control programs in Kenya.

Methodology and data: The study adopted the Economic Surplus Modeling approach, commonly used for the evaluation of commodity-related technological progress in agriculture (Alston *et al.*, 1995). The economic surplus model estimates the aggregate total monetary benefits for socio-economic agents involved in the introduction of a research innovation of development intervention in a targeted social environment (Maredia *et al.*, 2000). The approach was developed following the framework presented by Alston *et al.* (1995) and others under the assumptions of close economy, linear curves of supply and demand and parallel shift of the supply due to the introduction of BC. Producer and consumer annual surplus changes were calculated using the supply-shift parameter and times series-data of production, prices, and price elasticity supply and demand of maize and sorghum. The supply-shift parameter was calculated using the yield gain from the BC, the annual area covered by the BC, the maize and sorghum price elasticity of supply. Yield gain data was obtained from exclusion experiments while the annual covered area was obtained through a GIS model based on the dispersal rate of the released natural enemies and the coordinates of the release locations, assuming a concentric circle spread from the release points. The Net Present Value (NPV) was calculated by comparing the present total surplus to the present cost of the program using a discount rate of 10%. The Internal Rate of Return (IRR) and the Benefit-Cost Ratio (BCR) were then computed to appreciate the BC program efficiency. The equivalent poverty reduction was assessed following Alene *et al.*, (2009) using data on annual agricultural gross domestic product (AgDP), the annual poverty incidence and poverty elasticity.

Results: Findings of the welfare gain from the economic surplus model show that icipe BC intervention has contributed to an aggregate value of $US 0.74 billion over 20 years (1993 to 2013), with 76.7% ($US 568.06 million) from maize and the remaining 23.29% ($US 172.45 million) from sorghum, implying that the program has induced a highly positive impact on welfare in Kenya. Distribution of the welfare results on the same period provide evidence of higher surplus gain for producers ($US 424.80 million; 57.36%) compared to consumers ($US 315.70 million; 42.63%), the former mainly gain from yield loss reduction and then productivity-effect while the later gain from reduction in price due to the supply-shift. The total NPV over the analysis period was estimated at $US 108.80 million for maize, $US 45.63 million for sorghum cumulating at $US 141.52 for both crops. These estimates indicated that the total discounted benefits from the BC implementation far outweighed the total discounted cost of the program, thus justifying the higher profitability of investing in BC against stemborer attacks. One important step in this evaluation was to appreciate the efficiency of investment in the BC-research by calculating the internal rate to return on the investments. The overall IRR of 113.08% obtained for the aggregate crop is attractive because the return is above the prevailing discount rate considered of 10%. This result justifies that the investment in icipe's biological control research was a highly worthwhile investments. Efficiency analysis provide a BCR of 276:1 meaning that each dollar invested in the biological control program generates an additional higher value of 276 dollars for both crops. These benefits were higher than those obtained in other BC program evaluations in Africa, including De Groote *et al.* (2003) who estimated a BCR of 124:1 for the biological control program of water hyacinth in Southern Benin. Poverty impact expressed here as the proportion of poor people lifted out of poverty ranged from

0.01% in 1996 to 0.56% in 2013, averaging 0.35% per year and representing 57.40 thousands of poor moved from poverty annually with the implementation of BC (Midingoyi *et al.*, 2016).

Conclusion and Policy Implications: Using the economic surplus model, the resulting welfare change in terms of monetary surplus for both producers and consumers confirmed the positive impact of the BC program on social welfare. The estimation of the internal rate of return and the BCRs revealed the high efficiency of funds invested in BC research. The NPV also confirmed the high profitability of this investment. Moreover, the results showed yearly increases in the number of persons lifted out of poverty. This reveals that the BC intervention remains an important policy tool that can promote poverty reduction. Investment in promotion and up-scaling of the biological program against stemborers pests for sustained cereal production and poverty reduction in East and Southern Africa is recommended.

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13.4 Socio-economic Impacts and Extension Process of Conservation Biological Control in Mango Orchards in Réunion Island

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In Réunion Island, mango trees are confronted with a variety of pests (Miridae, Tephritidae, Cecidomyiidae, Thripidae, Coccidae, Acari) and diseases (bacteriosis, anthracnose, oidium) (Amouroux and Normand, 2013). This has led, over the years, to the increasing use of pesticides, in particular insecticides and herbicides. This agrochemical protection has its limitations: reduced insecticide efficacy on pests, weed resistance to herbicides, negative effects on the environment (soil, water) and on biodiversity (animals and plants) on an island internationally recognised as a biodiversity hotspot (Myers, 2003). There are also health risks for producers and consumers and ecological imbalances.

To replace this conventional agrochemical protection, an agroecological crop protection experience, based on conservation biological control (Deguine *et al.*, 2017), was initiated in Réunion Island in mango orchards in 2010. After two years of co-design with a large number of agricultural stakeholders, a Research and Development project, Biophyto, tested agroecological crop protection principles between 2012 and 2014 in a network of 12 pilot orchards. After this experimental phase, the dissemination of conservation biological control practices to other mango producers was carried out via transfer agencies and the government. Our study aims at presenting the main socio-economic results of the agroecological management of mango orchards in Réunion Island based on conservation biological control.

The Biophyto project: a conservation biological control experiment in conjunction with growers: The Biophyto project brought together a network of farmers and agricultural actors (research, experimentation, advisory, training, education, transfer, public authorities) with the aim of implementing agroecological practices using the principles of conservation biological control (Ferron and Deguine, 2005). These practices have enabled insecticide and herbicide treatments to be eliminated via the establishment of permanent plant cover in orchards to promote functional biodiversity below the mango canopy. In this project, it was possible to compare the performance of conventional (agrochemical) and agroecological orchards. The results of this experiment are very encouraging and may be useful to

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understand the ecological functioning of other tropical crops (http://www.agriculture-biodiversite-oi.org/Biophyto).

The farmers involved in the Biophyto project all adopted agroecological practices to a certain degree. Overall, these practices have proven to be effective in the promotion of plant health, in preserving the environment, adapted to the context, and easy to implement.

The role of biodiversity in the ecological functioning of mango orchards has been thoroughly studied and original knowledge about it has been acquired (Jacquot, 2017). As an illustration, over 120,000 arthropods were collected in orchards including nearly 800 morpho-species. Amongst these species, only a few can be considered as mango pests, while nearly 200 belong to the parasitoid guild.

During the Biophyto project, growers were informed and trained about the important role of functional biodiversity and the effectiveness of conservation biological control. With the use of agroecological practices (permanent vegetation cover, stopping insecticide and herbicide treatments), growers became aware of the existence and the important role of many other trophic groups (weeds, detritus, herbivores, detritivores, predators, parasitoids, pollinators, hyperparasitoids, etc.). They realised that the vast majority of arthropods present in their orchards were not harmful to mango trees, and that they were important for the stability of ecological balances.

During the three years of the project, insecticide and herbicide treatments were greatly reduced, and in most cases completely eliminated. At the same time, production costs were reduced by 35%. Overall, there were no production losses between agrochemical and agroecological plots except in rare instances where gall midge attacks were observed on a vulnerable mango variety. The added economic value was particularly appreciated by growers and was one of the reasons why the practices proposed were quickly applied in all orchards of the project during and after the Biophyto project.

After Biophyto: the transfer of practices by agencies and authorities: After the Biophyto project, the organizations for agricultural transfer (Association of Producers, Chamber of Agriculture) became involved and were accompanied by the authorities so that conservation biological control practices could be promoted and disseminated. Several development tools were created and implemented. First, a reference network of demonstration plots named "DEPHY FERME MANGUE" was set up in Réunion Island. Numerous visits were organized for mango growers so that they could experience a first approach of the agroecological practices. Secondly, an agricultural and environmental measure was set up to encourage producers to commit themselves to the agroecological protection of orchards. They benefitted from a grant of 900€/ha per year over a period of 5 years, providing that a permanent vegetation cover was maintained on the entire orchard (including flower strips on at least 5% of the farm). Thirdly, since conservation biological control is much more effective when it is used at a large scale like a production basin, an Economic and Environmental Interest Group was created at the request of mango growers from the main production area in Réunion Island, with the aim of establishing collective actions (including the management of agroecological practices).

Moreover, in Réunion Island, there is a general interest in the consumption of organic products. Since the Biophyto project, many mango producers have converted to organic farming. This move has been financially supported by the public authorities.

Finally, this agroecological experiment gave rise to a university-accredited diploma designed and implemented for growers and agricultural professionals. A professional qualification University certificate (CUQP), entitled "Agroecological Crop Protection" and awarded by the University of Réunion was designed with partners. Professionals who have graduated then act as leaders in the field and contribute effectively to the transfer and dissemination of conservation biological control practices.

Conclusion: After successful trials in Réunion Island on vegetable crops (more than half of the chayote production is organic in 2017 compared to 0% in 2009), agroecological protection based on conservation biological control was applied to mango orchards. This experience shows that an agroecological approach can be adopted in a producer environment while maintaining or even improving socio-economic performance. Beyond these socio-economic aspects, the positive impacts of conservation biological control on the environment and on human health became clear. Different professionals, including growers, are also becoming aware that conservation biological control practices contribute significantly to the ecological sustainability of agroecosystems. Other experiments are in process in Réunion and in the neighbouring countries of the Indian Ocean.

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13.5 Chronicling the Socio-economic Impact of Integrating Biological Control, Technology, and Knowledge over 25 Years of IPM in Arizona

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Integrated Pest Management (IPM) in Arizona cotton over the last 25 years has depended on the successful integration of biological and chemical controls along with other insect management knowledge and technologies. Contemporaneous measurement systems enabled economic evaluations with long-term datasets to examine economic outcomes and impacts, as well as durability of adopted technology (Ellsworth *et al.*, 2007). Despite devastating outbreaks of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), and an invasive whitefly, *Bemisia tabaci* (Gennadius) (Middle East Asia Minor 1; Dinsdale *et al.*, 2010) [= *B. argentifolii* (Bellows *et al.,* 1994)] (Hemiptera: Aleyrodidae), in the early 1990s, major economic losses to the plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), in the late 1990s, and continuing pest threats throughout the last two decades, cotton growers in Arizona have saved >US$500 million through uptake and adoption of technology, and knowledge to implement developed IPM plans that include conservation biological control (CBC) as a major component.

CBC as a tactical component of IPM was specifically developed for and directly supports the integrated management of whiteflies (Ellsworth and Martinez-Carrillo, 2001; Ellsworth *et al.,* 2006; Naranjo and Ellsworth, 2009a,b). However, integrated use of CBC has also prevented pest resurgences of other key pests and outbreaks of many secondary pests. CBC, as an actively considered practice by pest managers, became possible only through the successful discovery, development and implementation of selective control tactics that were effective against each of the three key pests of this system. Cotton genetically engineered to express a lepidopteran-active toxic protein from *Bacillus thuringiensis* Berliner (Bacillicaeae) was successfully introduced in 1996 for the direct control of pink bollworm (Naranjo and Ellsworth, 2010; Tabashnik *et al.*, 2010). At the same time, two whitefly-specific insect growth regulators (IGRs, buprofezin and pyriproxyfen) were introduced along with key knowledge domains (e.g., stage specific sampling, thresholds, chemical use and resistance management plans) that enabled selective control of whiteflies. Ten years later, a *Lygus*-specific feeding inhibitor (flonicamid) was introduced for the selective control of this third key pest (Ellsworth and Barkley, 2005), and grower collaboration to eradicate the pink bollworm was initiated.

CBC is an integral component of a larger multi-pest IPM system, making the parsing out of its effect on the system challenging. Moreover, uptake by producers is largely governed by the successful adoption of host plant resistance and chemical controls

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that permit CBC to function optimally. Thus, calculation of the economic savings due to these hard technologies, Bt cotton in 1996, whitefly IGRs in 1996, and *Lygus* feeding inhibitor in 2006, can be estimated more directly by examining pre- and post-introduction data for those respective periods (pre-/post-1996 or pre-/post-2006). For example, gains in foliar insecticide costs and in saved yield related to pink bollworm technologies pre- and post-1996 and pre- and post-2006 were US$100/ha and US$63/ha after the introduction of Bt cotton and pink bollworm eradication program efforts, respectively. These changes together with all other advances in key and other pest control also contributed to the > 90% reduction in broad-spectrum insecticide use in Arizona cotton since 1996.

This major change in insecticide use patterns enabled opportunities for generalist predators and other natural enemies to thrive and estimating their value is based on examining the remaining changes not directly associated with the introduction of one of the previously mentioned hard technologies. In 2006, US$91/ha was saved in whitefly management, despite there being no specific or major additional hard technologies introduced during this period targeting whitefly. This gain was likely due to the higher functioning of CBC and natural control of whiteflies after the remaining broad-spectrum controls in the system were replaced by the selective *Lygus* feeding inhibitor in 2006. Likewise, there were savings of US$26/ha after 1996 in management of all "other" secondary pests, even though there were no specific hard technologies deployed against them during this period. This, too, was likely due to the CBC enabled by the replacement of broad-spectrum chemistries previously used against pink bollworm and whiteflies. Thus, the cumulative benefit of increased CBC during this 20 year period can be placed conservatively at US$117/ha ($26 + $91). This represents economic gains that approach half of the total measured savings to growers since 1996 (total through 2016 = US$274/ha inclusive of trait technology grower costs) and is closely aligned with other Arizona pest manager estimates of the value of biological control in their cotton system (US$108/ha; Naranjo *et al.*, 2015).

The Arizona IPM model resulted in broad-scale improvements to the environment and the economy. CBC enabled by strategic deployment of critical hard technology (Bt cotton, whitefly IGRs, *Lygus* feeding inhibitor) and soft technology (knowledge gains in sampling, thresholds, resistance management, each part of the Arizona IPM strategy) have produced a highly selective, safe, and economically and ecologically sustainable IPM system.

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Session 14: Marketplace II – Free Topics

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14.1 Do GM Plants with Stacked Insecticidal Traits Pose an Increased Risk to Biological Control?

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Genetically modified (GM) cotton, maize, and soybean varieties that produced insecticidal Cry and/or VIP proteins derived from *Bacillus thuringiensis* (Bt) were grown on a total of 98.5 million hectares worldwide in 2016 (ISAAA, 2016). Bt-transgenic plants have thus become an important pest management tool in those important field crops. While the early Bt-transgenic plants produced only single Cry proteins (e.g., Cry1Ab in maize and Cry1Ac in cotton), modern products contain several insecticidal traits (ISAAA, 2016). This approach of combining (stacking) different insecticidal traits has the advantage that it provides protection from a larger pest spectrum and that it reduces the potential of evolving resistance in the target pests (Taverniers *et al.*, 2008; Que *et al.*, 2010). SmartStax® maize for example produces Cry1A.105, Cry1F, and Cry2Ab2 with activity against Lepidoptera pests such as the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) and Cry3Bb1, Cry34Ab1, and Cry35Ab1 to control Coleoptera pests such as *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae).

One concern with the growing of Bt crops is that they could cause harm to valued non-target organisms including natural enemies that help to control herbivores in crop fields and thus contribute to sustainable pest management. The risk to those valued non-target organisms and the ecosystem service they provide is thus assessed in the environmental risk assessment that precedes the cultivation of any GM plant (Romeis *et al.*, 2008b). To assess the risk both the toxicity of the produced insecticidal compounds (hazard) as well as the concentrations at which they are exposed in the field (exposure) are taken into account.

Many years of study have not revealed evidence of direct Cry protein effects on non-target species outside of the target insect order (Naranjo, 2009). Occasionally reported adverse effects could not be verified and were likely caused by poor study design (Shelton *et al.*, 2009; Romeis *et al.*, 2013). Consequently, today's Bt-transgenic crops are highly compatible with biological control (Romeis *et al.,* 2006, 2008a). This together with the fact that Bt-transgenic varieties have reduced the use of chemical insecticides (NASEM, 2016) has increased in-field biodiversity and biological control (Lu *et al.*, 2012).

The current environmental risk assessment approach has been challenged by the increasing number of GM plants with stacked traits that are submitted for regulatory approval (De Schrijver *et al.*, 2007; Taverniers *et al.*, 2008). In the European Union, GM plants with stacked traits are regarded as a novel product that requires a full dossier including results from non-target studies. In other jurisdictions (e.g., USA), combinations of approved traits obtained by conventional breeding can be regulated based on the assessment of the individual traits when three conditions are satisfied: (i) the presence and

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the structure of the inserted material have been conserved in the stacked product, (ii) when the expression of the insecticidal compound in the parental lines and the stack is comparable, and (iii) when the different insecticidal compounds do not act in a synergistic way (Levine *et al.*, 2016). The latter is assessed in laboratory studies using artificial diets and purified compounds using the target pest or any other sensitive insect species (Raybould *et al.*, 2012; Levine *et al.*, 2016). This approach has been criticised by some which argue that the different Cry proteins (or other insecticidal compounds) in the food of herbivores and predators could interact in a way that may lead to unexpected and unpredictable effects on non-target species, even if they are not susceptible to the individual compounds (Hilbeck and Otto, 2015).

We have thus assessed the impact of SmartStax® maize containing a total of six Cry proteins on three predators, i.e., *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), and *Phylloneta impressa* (L. Koch) (Araneae: Theridiidae) (Svobodová *et al.*, 2017). We have conducted bi- and tri-trophic experiments in the laboratory using maize pollen, or maize-fed aphids, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae), or spider mites, *Tetranychus urticae* Koch (Acari, Prostigmata: Tetranychidae), as food. For all three predators, no adverse effects in the Bt maize treatment on survival and on sublethal endpoints such as development and weight were observed when compared to non-Bt maize treatments. Analyses by DAS-ELISA (double antibody sandwich enzyme linked immunosorbent assay) confirmed the uptake of the Cry proteins by the predators. Predators contained lower Cry protein concentrations than their food. Among the different predators, ladybeetle larvae showed higher concentrations than lacewing larvae and juvenile spiders. Consequently, our study provides evidence that the Cry proteins do not interact in a way that poses a risk to the tested non-target species.

Such a lack of effects in stacked Bt crops or artificial diets containing multiple Cry proteins has been reported for other arthropod predators (Li *et al.*, 2011; Tian *et al.*, 2013, 2014; Su *et al.*, 2015; Guo *et al.*, 2016). It thus appears that GM plants with stacked insecticidal traits do not pose any greater risk to non-target beneficial species compared to the single trait plants and that the risk assessment approach that is investigating the synergistic effects of the combined insecticidal compounds using sensitive species is sufficiently robust.

Given that stacked GM plants provide a better pest control and delay the evolution of resistance, they are likely to be used increasingly, which may further reduce the use of chemical insecticids and thus benefit biocontrol.

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14.2 Initial Evaluation of Two Native Egg Parasitoids for the Control of *Bagrada hilaris*, an Invasive Stink Bug in Western USA

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First reported in California in 2008, *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), a major pest of Brassicaceae, has now spread southeastward into several US states and Mexico (Palumbo *et al.*, 2016; Reed *et al.*, 2013; Sanchez-Pena, 2014). The supposedly native distribution of *B. hilaris* ranges from South Africa to Southeast Asia where it is also a crop pest (Gunn, 1918). This highly polyphagous stinkbug feeds on many families but is especially damaging to cole crop species in the Brassicaceae on which it is a serious pest in both the introduced and native range (Palumbo *et al.*, 2016). A classical biological control project has recently begun. We present here a preliminary phylogeography study to determine the likely origin of the pest, and a study of the effect of host quality on parasitism by two egg parasitoids from Pakistan (Mahmood *et al.*, 2015).

Genetic evaluation: As an initial step, collections of native and invasive bagrada populations were genetically screened for pinpointing the geographic origin of the invasive North American populations. Adult specimens were either field collected or obtained from lab colonies, and stored in absolute alcohol at 4°C until DNA extraction. The Barcode region *CO1* was sequenced for 16 populations from four countries. This barcode dataset was merged with sequences retrieved from Genbank and BOLD. Preliminary data (Fig. 14.1.1) show that all bagrada in the USA/Mexico belong to one haplotype, which is closely related to the most frequent haplotype evidenced in Pakistan. Specimens from Kenya, South Africa, and Morocco are genetically more distantly related to the American specimens.

Native natural enemies: Concomitantly, surveys in Pakistan in 2016, were conducted by using sentinel Bagrada eggs to collect native parasitoids (Mahmood *et al.*, 2015). It led to collection of two egg parasitoid wasps identified as *Trissolcus hyalinipennis* Rajmohana & Narendran and *Gryon* sp. (Hymenoptera: Platygastridae) by E.J. Talamas (Systematic Entomology Laboratory, Washington, District of Columbia, USA).

Biological evaluation: We established a laboratory colony of each parasitoid species in our quarantine and addressed the question what the effect of age of the host egg had on suitability for parasitism. Host eggs were collected daily and held for different durations to

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provide eggs of different ages, from 0-1 to 3-4 days old. In addition, some 0-1-day-old eggs were frozen at -80°C. We exposed female parasitoids (20 replicates for *Gryon* sp. and 16 for *T. hyalinipennis*) to 6 *B. hilaris* eggs for 1 h at 22°C. Only one age class of eggs was exposed to parasitoids at a time (no-choice). We observed that both *Gryon* sp and *T. hyalinipennis* successfully parasitized live host eggs, regardless of their age up to 4-days-old (Fig. 14.2.2). However, frozen eggs were much less suitable. *Trissolcus hyalinipennis* was able to complete development but oviposited significantly less than on non-frozen eggs. On the other hand, *Gryon* sp, mostly rejected frozen eggs, but was occasionally able to complete full development. These results indicate that both parasitoid species can successfully attack host eggs up to 4 days old, and possibly older. However, frozen eggs generally are not suitable under our conditions. This means that the use of frozen eggs for sentinel eggs to survey for parasitoids in the field would probably not detect either of these parasitoid species. Both parasitoid species have been provided to cooperators in the USA to conduct host specificity studies.

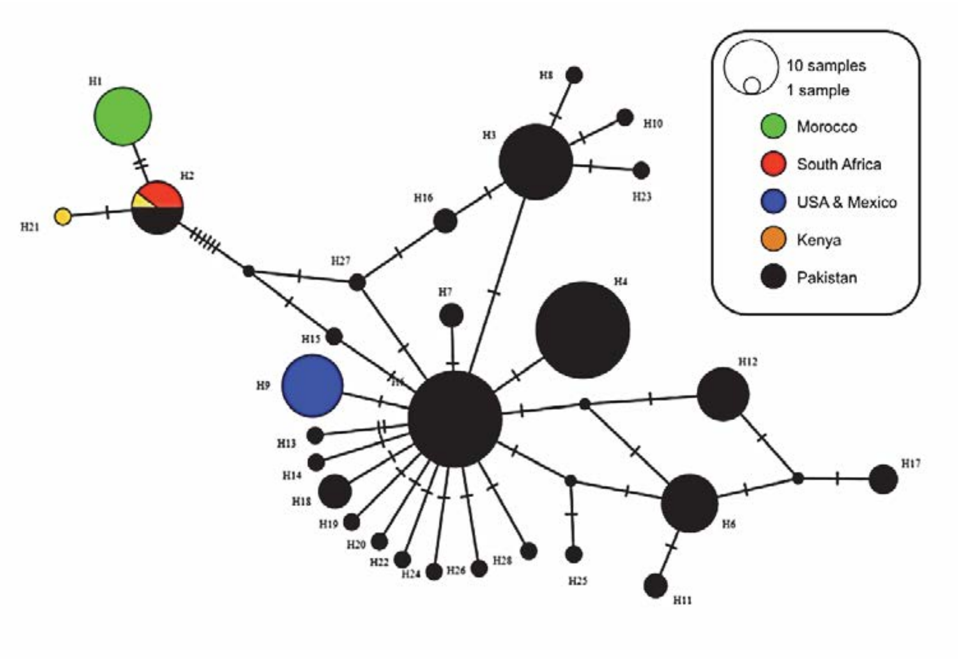
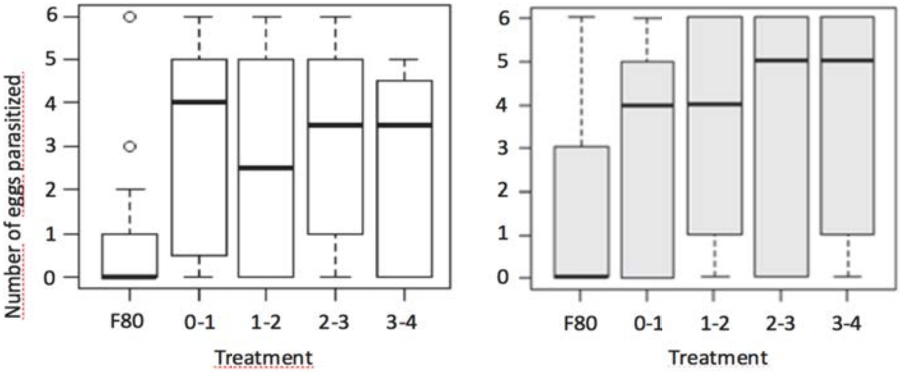


Fig. 14.2.1. Evolutionary network depicting genetic relatedness among barcode haplotypes recovered from *Bagrada hilaris*. Each circle represents a different haplotype, the colour corresponds to country, the diameter is proportional to abundance, and the cross hatches *indicate the number of mutations (nucleotide changes) between haplotypes.*

Future directions will aim to understand the foraging capacity of egg parasitoids, especially with eggs buried into the soil by bagrada females. The overall success of this classical biocontrol programme will emerge from close collaborations with research institutes in the native range of Bagrada such as CABI Pakistan, ARC, South Africa, and Kalro, Kenya.

Fig. 14.2.2. Parasitism rate by *Gryon* sp (white) and *Trissolcus hyalinipennis* (grey) using live bagrada eggs from 0-1 days to 3-4 days old or frozen 0-1-day-old eggs (F80). Edges of each box plot represent the first and the third quartiles; the central lines in bold, the medians; the dashed lines, the maxima and minima of 95% of the values and the white circles the outliers.



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14.3 Old and New Host-parasitoid Associations: Parasitism of the Native African and Invasive Fruit flies Species

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In Africa, horticulture is an important agricultural subsector for ensuring food security and providing job opportunities for millions of growers and other stakeholders along the commodities value chain. However, the subsector is hindered by several constraints, ranked high among them is the tephritid fruit flies, causing both direct (> 80% fruit yield losses) and indirect losses through quarantine restriction imposed by importing countries, resulting in annual losses of approximately two billion dollars (Ekesi *et al.*, 2016). Although Africa is home of key notorious fruit fly species, some of which, e.g., *Ceratitis capitata* Wiedemann and *Bactrocera oleae* Rossi (Diptera: Tephritidae), invaded and established as a major pests in other part of the world (De Meyer and Ekesi, 2016), the continent continues to be invaded by alien fruit flies (Lux *et al.*, 2003; Mwatawala *et al.*, 2007; De Meyer and Ekesi, 2016) further compounding the threat to horticultural industry in Africa. Proper management of these pests requires a holistic IPM approach of which biological control is viewed as an essential component (Wharton, 1989; Mohamed *et al.*, 2016). This paper highlights the results of performance of three African native parasitoid species; *Psyttalia concolor* (Szépligeti), *P. cosyrae* (Wilkinson) (Hymenoptera: Braconidae) and *Tetrastichus giffardii* Silvestri (Hymenoptera: Eulophidae) and two exotic introduced parasitoids, *Fopius arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) against six native tephritid fruit fly species, *Ceratitis capitata* (Wiedmann), *C. cosyra* (Walker), *C. rosa* (Karsch), *C. fasciventris* (Bezzi), *C. anonae* Graham and *Dacus ciliatus* Loew, in addition to two alien invasive species *Bactrocera dorsalis* (Hendel) and *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae). *Fopius arisanus* and *D. longicaudata* were introduced from Hawaii for classical biological control of *B. dorsalis* following its invasion and wide spread into Africa (Mohamed *et al.*, 2008; 2010).

Methodology: The acceptability of the different species of fruit flies by each parasitoid species was evaluated in no choice test. After 3 hours of exposure of 10 individuals of the suitable host stage (eggs for *F. arisanus*; or larvae for *P. concolor*, *P. cosyrae*, *T. giffardii* and *D. longicaudata*) to the parasitoids, the hosts were dissected in a saline solution under microscope. Then, the numbers of parasitized egg/larvae were recorded.

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To further investigate the performance of these parasitoid species on the target fruit fly species, the suitable host stages were exposed to the parasitoid in the same way described above. However, after the exposure the hosts, they were maintained till fruit flies and parasitoid emergence. Number of enclosed parasitoid wasps were counted and computed as percentage out the total number of retrieved puparia of each host and each parasitoid species.

Results: Acceptability in terms of the number of parasitized eggs or larvae varied with the host species, generally being highest on the parasitoid natural host. However, the invasive fruit fly *B. dorsalis* was moderately accepted by the indigenous parasitoid *T. giffardii* while the native fruit fly, *C. cosyra* was highly accepted by the introduced parasitoid *D. longicaudata* (Table 14.3.1).

Table 14.3.1. Acceptability of the five parasitoid species for key African native and invasive fruit flies species.

Host species	Host species				
	Psyttlia concolor	***Psyllia cosyrae***	***Tetrastichus giffardii***	***Fopius arisanus***	***Diachasmimorpha longicaudata***
Ceratitis capitata	++	+	+	+	+
Ceratitis cosyra	+	++	+	+	++
Ceratitis rosa	-	-	-	-	-
Ceratitis fasciventris	-	-	-	-	-
Ceratitis anonae	-	-	-	-	-
Dacus ciliatus	N/T	N/T	++	N/T	N/T
Bactrocera dorsalis	-	-	+	++	++
Zeugodacus	-	-	-	N/T	N/T

Poorly accepted (-); Moderately accepted (+); Highly accepted (++); Not Tested (N/T).

Generally, parasitoid performance, as measured by percent viable eclosed parasitoid wasps, was higher when reared on their respective co-evolved host species (Fig. 14.3.1 and 14.3.2). Although, the native parasitoid also perfomed differentially on the native fruit flies species (Fig. 14.3.1) with species in the FAR group (*C. rosa*, *C. fasciventris, C. anonae*) being non or very poor hosts. The introduced parasitoids were able to establish new association with some of the native fruit fly species, but the reverse was not true; whereby, the native parasitoid were unable to parasitize the alien fruit fly species as their egg were encapsulated in these host species (Fig. 14.3.1 and 14.3.2).

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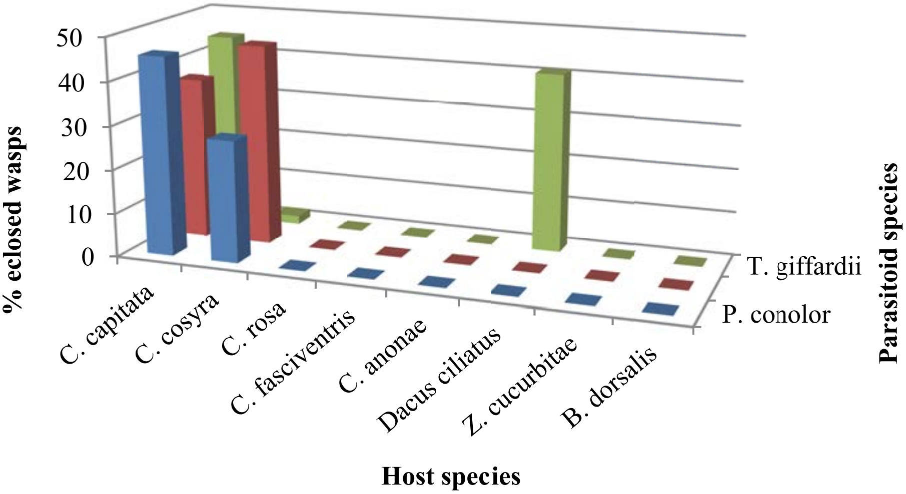

Fig. 14.3.1. Performance of the native parasitoid species, *Tetrastichus giffardii* and *Psyttalia concolor*, on native and invasive fruit flies species.

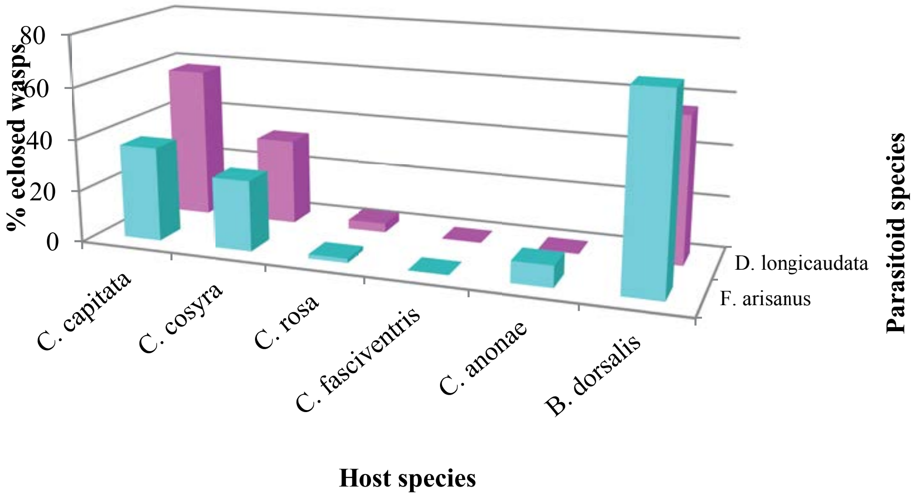

Fig.14.3.2. Performance of the introduced parasitoid species, *Diachasmimorpha longicaudata* and *Fopius arisanus*, on native and invasive fruit flies species.

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14.4 Harnessing of Floral and Faunal Biodiversity of Rice Ecosystems for Pest Management

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Rice paddies have a complexity of fauna which interact with each other and provide ample scope for natural biological control (Kiritani, 2000). Some simple techniques of IPM can help to conserve natural enemies and enhance pest control. Increasing floral diversity or ecological engineering can be an important strategy for conservation of natural enemies (Gurr, 2009).

Ecological engineering aims at habitat manipulation for conservation of native natural enemies. Field and laboratory studies were undertaken at the research farm of ICAR - Indian Institute of Rice Research (IIRR), Hyderabad and farmer's field at Nalgonda district of Telangana state, India. The treatments were (i) organic manuring, (ii) alleyways of 30 cm every 2 m, (iii) monitoring of stem borer with pheromones, and (iv) increasing floral diversity by growing a border crop in rice paddies on biodiversity of beneficial insects. Border crops tested were *Tagetus erecta* L. (Asteraceae) (Fig. 14.4.1), *Vigna unguiculata* L., *V. mungo* (L.) Hepper, *V. radiata* (L.) R. Wilczek, *Crotalaria juncea* L. (Fabaceae), *Coriandrum sativum* L. (Apiaceae), and *Abelmoschus esculentus* (L.) Moench (Malvaceae).

Fig. 14.4.1. *Tagetus erecta* grown as bund crop in rice paddies.

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Parasitization of hoppers, particularly the brown plant hopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae), a key pest of rice, was assessed by baiting studies. Potted plants exposed to gravid females of the brown planthopper for 48 hrs were placed in the field at varying distances of 1, 2 and 3 m from flowering borders for 3 days. The plants were then brought to the laboratory and maintained for a week until develoment of parasitoids. The eggs were dissected out and parasitism was determined based on the colour of the eggs i.e., translucent white for healthy eggs, lemon yellow for eggs parasitized by *Oligosita* sp. (Hymenoptera: Trichogrammatidae) and brick red for eggs parasitized by *Anagrus* sp. (Hymenoptera: Mymaridae). A set of pots were also allowed for emergence of parasitoids to confirm the species. The predominat parasitoid was *Oligosita* sp., followed by *Anagrus* sp.

Planting of flowering plants on rice bunds had significant impact on biodiversity and parasitization rates. Significant differences were observed in parasitism of hopper eggs in plots with different crop borders (Fig. 14.4.2) in comparison to plots without flower borders (F= 4.91; df 7; p <0.01). It ranged from 10-50% between treatment plots and the mean highest parasitism of hopper eggs was observed near a bund of black gram (45.6 %) while the lowest was observed in farmer's practice plots without flower border (11.4%). The flowers on bunds were found to increase the longevity and fecundity of hopper egg parasitoids. Floral nectar contains not only sugars but are a source of proteins, amino acids, lipids and many other organic and inorganic substances (Hiel, 2011). Nectar feeding can increase the survival of parasitoids multi-fold, enhance their fecundity and increase their general reproductive fitness (Zhu *et al.*, 2013).

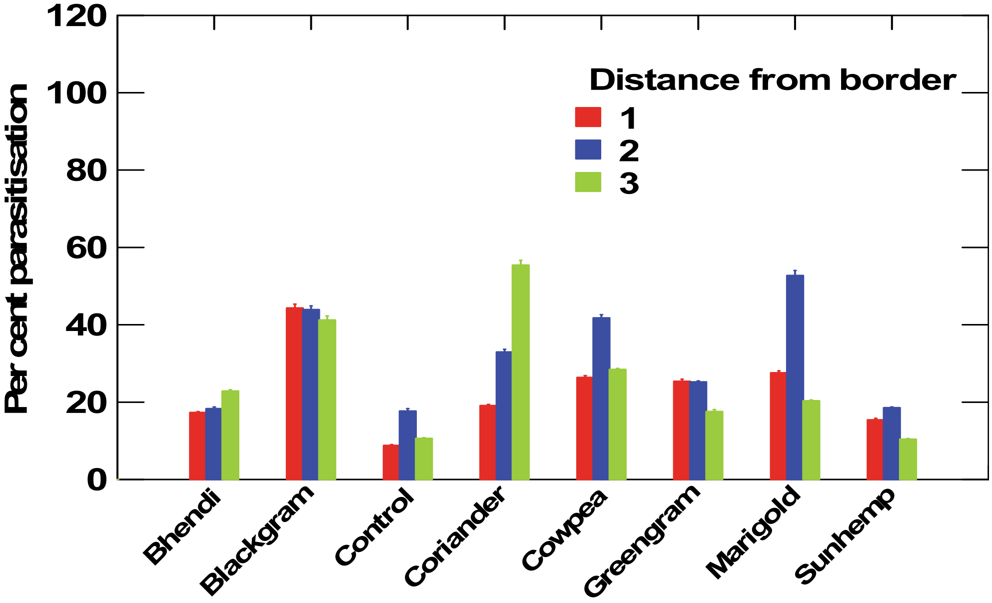

Fig. 14.4.2. Parasitization of hopper eggs in farmer's field by egg baiting technique.

Apanteles sp., *Bracon* sp. (Hymenoptera: Braconidae), *Gonatocerus* sp., *Mymar taprobanicum* Ward, *Anagrus* sp. (Hymenoptera: Mymaridae), and *Tetrastichus schoenobii* Ferrière (Hymenoptera: Eulophidae) were some of the parasitoids observed in yellow pan traps placed near the flowering borders. Intelligent harnessing of floral and faunal biodiversity provides a novel ecofriendly opportunity of managing rice pests with additional economic benefits, particularly for resource-poor Asian farmers, along with safety to human health and environment.

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14.5 Population dynamics of *Plutella xylostella* (Lepidoptera: Plutellidae) and Its Parasitoids Along Altitudinal Gradients of the Eastern Afromontane

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Diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae), is a major insect pest of crucifer crops, and it is resistant to synthetic insecticides. Parasitoid species provide the most effective control of DBM populations. However, weather requirements vary between species and populations and may modulate the survival, abundance, diversity and movement of species. Studying altitudinal gradients provides an alternative to setting up experiments when investigating climate change and thus, assessing the seasonal abundance of DBM and diversity of its parasitoids along Mount Kilimanjaro and Taita hills provided new insights on sustainability of current biological systems across the year for new pest management strategies.

Methods: Monthly field surveys of DBM and its parasitoids were conducted on twenty-four crucifer vegetable farms spread across three altitudinal transects of the Eastern Afromontane regional of Mt. Kilimanjaro (700 - 1690 meters above sea level (masl)) and Taita hills (700 - 1785 masl), from December 2012 to February 2014. The transects were sub-divided into three altitudinal zones: low (700 - 1,200 masl), medium (1,201 - 1,600 masl) and high (> 1,600 masl). The total numbers of larvae and pupae found on each plant were counted and recorded. The larvae and pupae collected from individual farms were packed into plastic containers and observed for parasitism in the laboratory. The parasitoids were identified at icipe, using dichotomous identification keys (Goulet and Huber, 1993; Azidah *et al.*, 2000) and interactive keys from Sharkey (2017). The samples of *Cotesia* sp. collected in Mt. Kilimanjaro and Taita hills were subjected to barcoding using LCO 1490/HCO 2198 primers (Folmer *et al.*, 1994) for confirmation of the identity.

Diamondback moth abundance: Distribution of the DBM population varied in the two altitudinal transects. The abundance was four times higher during the short rains as compared to the other seasons and was least during the long rainy season due to the mortality effect of rains ($\chi^2 = 37.82$, df = 3, $P < 0.001$; negative binomial GLM) (Table 14.5.1) (Iga, 1985; Kobori and Amano, 2003). Rainfall may have interfered with adult

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flight and mating activities which slowed the population growth. However, the increase in pest status during short rains might be probably due to longer intervals of dry spells between rains that could have allowed population build up. Along the altitudinal gradient, the DBM population increased in the high zones as compared to medium and low zones. The DBM is known to prefer temperatures < 20°C than higher temperatures for survival and growth (Wakisaka *et al.*, 1992; Liu *et al.*, 2002), as was evident in the high zones in the present study. Marchioro and Forester (2011) observed highest survival and oviposition of DBM at 20°C, which is in line with this study outcome.

Table 14.5.1. Seasonal abundance of DBM in cultivated crucifers along the three altitudinal zones.

		Number of DBM/plant		
Transect	**Season**	**Low zone**	**Medium zone**	**High zone**
Mt. Kilimanjaro	Hot dry	0.63 ± 0.12	0.37 ± 0.11	0.52 ± 0.14
	Long rains	0.25 ± 0.12	0.14 ± 0.05	0.17 ± 0.09
	Cold dry	0.16 ± 0.07	0.41 ± 0.06	0.32 ± 0.08
	Short rains	0.43 ± 0.22	0.3 ± 0.07	0.71 ± 0.14
	Annual average	0.38 ± 0.08	0.32 ± 0.04	0.47 ± 0.07
Taita hills	Hot dry	0.30 ± 0.06	0.28 ± 0.06	0.56 ± 0.08
	Long rains	0.03 ± 0.02	0.19 ± 0.07	0.26 ± 0.21
	Cold dry	0.31 ± 0.08	0.30 ± 0.06	0.39 ± 0.09
	Short rains	0.84 ± 0.48	0.80 ± 0.14	0.59 ± 0.13
	Annual average	0.41 ± 0.13	0.41 ± 0.05	0.49 ± 0.06

Parasitoid abundance and diversity: Parasitoid species such as *Diadegma semiclausum* (Hellen), *Diadegma mollipla* Holmgren, *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae), *Cotesia vestalis* Haliday, *Apanteles sp*., *Apanteles aethiopicus* Wilkinson (Hymenoptera: Braconidae), *Omyzus sokolowskii* (Kurdjimov), (Hymenoptera: Eulophidae) and *Brachymeria sp*. (Hymenoptera: Chalcididae) emerged out of the parasitized DBM collected from both transects. Comparing the two transects, the level of parasitoid diversity was higher along Mt. Kilimanjaro (Fig. 14.5.1) because of the different sources of foods and host insects availed from the more structurally complex cropping system (Marino and Landis, 1996) characterized by the Chagga homegardens.

The homegardens also provides microclimate for wild crucifer species which offers an alternate refuge to both DBM and its parasitoids. Individual parasitoid species showed a more specific colonization range, with *C. vestalis* and *D. semiclausum* providing the most effective DBM parasitism in the low and high zones, respectively. The parasitoid diversity was also more diverse in the low zone due the hot and humid climate suitable for most parasitoids. However, this diversity decreased with increased altitude as the prevailing environmental conditions, particularly declining temperatures, seems to have favored the more competitive *D. semiclausum*.

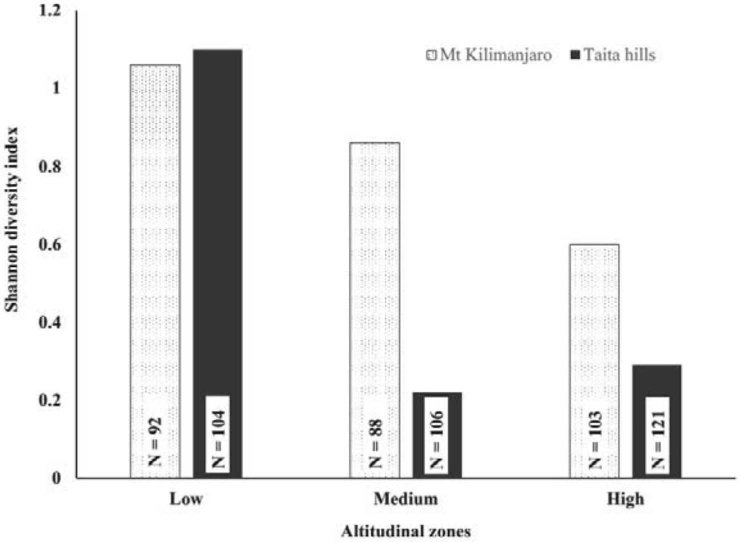

Fig. 14.5.1. Diversity of parasitoid species of DBM along the altitudinal zones of Mt. Kilimanjaro and Taita hills. N = number of samples.

The identity of *Cotesia* sp. collected in Mt. Kilimanjaro and Taita hills was confirmed as *C. vestalis* using molecular techniques. This indicates that *Cotesia vestalis* introduced to reinforce the DBM parasitism has successfully established and distributed in East Africa especially in the warmer areas in the low altitudes of Mt. Kilimanjaro and Taita hills.

Conclusion: Across the altitudinal gradient, the season abundance of DBM was found to be influenced by temperature and rainfall incidence. High altitude zones during short rainy season were found to be highly suitable for the growth and development of DBM. Cropping systems and abundance of host insects was found to significantly influence the diversity and abundance of DBM parasitoids in the Eastern Afromontane regions.

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Poster Presentations

Poster 1: Discovery of *Phasmarhabditis hermaphrodita* (Nematoda) in the USA and Its Potential Importance in the Biological Control of Invasive Gastropods

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Snails and slugs are among the most serious pests of agriculture. Control measures are focused on chemical molluscicides but their efficacy is very variable. In Europe, a commercially available biological control agent is used to help manage slugs in a range of crops. The active agent is a nematode called *Phasmarhabditis hermaphrodita* (A. Schneider) (Rhabditida: Rhabditidae) and its associated bacteria, *Moraxella osloensis* (Pseudomonadales: Moraxellaceae). Multiple past attempts at recovering *Phasmarhabditis* from slugs and snails in the US have been unsuccessful but we recently discovered *P. hermaphrodita* from a range of slug species in California and Oregon. This nematode has only been recovered from European invasive gastropod species in the US and not from native species, suggesting an accidental introduction. Virulence trials with this strain have shown that it is lethal to a range of pest slugs and snails highlighting its potential role as a biological control agent of these pests in the US.

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Poster 2: Feeding Behavior of *Rumina decollata* (Gastropoda) Raises Questions about Its Efficacy as a Biocontrol Agent of the Pestiferous Brown Garden Snail, *Cornu aspersum* (Gastropoda)

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The facultative predatory snail, *Rumina decollata* (L.) (Gastropoda: Subulinidae), was accidentally introduced to the US in the early 1800s (Batts, 1957) and it has been used as a biological control agent of *Cornu aspersum* (O. F. Müller) (Gastropoda: Helicidae) in Californian citrus for almost 50 years. Although there are reports of it apparently controlling *C. aspersum* in citrus groves, rarely, has direct causality been demonstrated experimentally (Fisher *et al.*, 1980; Fisher and Orth, 1985). In addition, the snail is a known pest of cultivated plants (Fisher and Orth, 1985) and vague statements regarding the size of *C. aspersum* that are vulnerable to predation are scattered throughout the scientific literature (Fisher and Orth, 1985; Sakovich, 2002). Also, since *R. decollata* is a generalist malacophage (Barker and Efford, 2002), there are concerns about non-target impacts of its use in biological control programs. The goals therefore of this research were to: 1) determine the size of *C. aspersum* vulnerable to predation by *R. decollata*; 2) determine whether *R. decollata* exhibits a preference for snail prey over a known plant food, and 3) assess the vulnerability of *C. aspersum* eggs to predation by *R. decollata*.

The maximum size of *C. aspersum* killed by *R. decollata* was determined using choice and no choice tests. Firstly, individual adult *R. decollata* (n=27) were provided with a choice of a small (10-14 mm shell diameter), medium (15-24 mm) and large (>24 mm) *C. aspersum*. Following these bioassays, a second set of choice tests were set up where individual adult *R. decollata* (n=9) were presented with a choice of a small (10-14 mm) and a very small *C. aspersum* (<10 mm) only. In choice tests with a known vegetable food, i.e., carrot root (Fisher *et al.*, 1980), individual *R. decollata* (n=30) were provided a small *C. aspersum* (3-13mm shell diameter) and a piece of carrot approximately the same weight as prey snails. These bioassays were conducted in polypropylene containers (13” x 6.5” x 4.75”) lined with a single layer of damp paper towel. For the ovicidal bioassays individual adult *R. decollata* (n=10) were presented with five fresh (<24 hrs of age) *C. aspersum* eggs placed in 5 cm diameter Petri dishes lined with damp filter paper. For all bioassays, observations were made after 24, 48, 72 hrs and 7 days.

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In choice tests with small, medium and large *C. aspersum*, only small snails within the size range 10-13 mm were consumed by *R. decollata* (Table P2.1). Furthermore, snails were only killed in 18.5% of the bioassays. In the second set of choice bioassays (small versus very small *C. aspersum*) five very small and one small snail were consumed (Table P2.1). In the replicate where the small snail was attacked, the very small snail was killed first. Very small snails were eaten in 55.6% of the bioassays. For no choice tests, *R. decollata* again only consumed snails in the smallest category and predation occurred in 50% of these replicates.

Table P2.1. Number of very small (<10mm)*, small (10-14mm), medium (15-24mm) and large (>24mm) *Cornu aspersum* killed by adult *Rumina decollata* in laboratory choice and no choice tests. Numbers in parenthesis are the actual sizes (shell diameter in mm) of prey snails killed. Table modified from McDonnell *et al.* (2016).

		Sample size	24hrs	48hrs	72hrs	7 days	Total Consumed
Choice test 1	Small	27	0	1 (12)	0	4 (10, 11, 11, 13)	5
	Medium	27	0	0	0	0	0
	Large	27	0	0	0	0	0
Choice test 2	Very small	9	3 (3, 6, 6)	1 (7)	1 (7)	0	5
	Small	9	0	0	1 (12)	0	1
No choice	Small*	12	1 (9)	1 (8)	0	4 (7, 8, 10, 7)	6
	Medium	12	0	0	0	0	0
	Large	12	0	0	0	0	0

* For no choice tests the small and very small categories were combined so that for small snails the size range was 3-14mm.

When individual *R. decollata* (n=30) were presented with a choice between a piece of carrot and a prey snail within its preferred size range, 93.33% (±4.22%) of test snails selected the former, 3.33% (±3.33%) selected *C. aspersum* and 3.33% (±3.33%) did not make a choice. In terms of egg consumption, the mean (+ SE) percentage of eggs (n=50) consumed per decollate snail was 62% (±10.09%), and 90% of snails had consumed at least two eggs after 7 days (Table P2.2).

Table P2.2. The total number, mean number (+ SE) and mean percentage (+ SE) of *Cornu aspersum* eggs consumed by ten individual *Rumina decollata* after 24hrs, 48hrs, 72hrs and 7 days. Each snail was presented a total of five eggs. Table modified from McDonnell *et al.* (2016).

	Egg predation (24hrs)	Egg predation (48hrs)	Egg predation (72hrs)	Egg predation (7 days)
Total	17	25	27	31
Mean No. ± SE	1.70 ± 0.40	2.50 ± 0.52	2.70 ± 0.52	3.10 ± 0.50
Mean % ± SE	34.00 ±7.92	50.00 ± 10.44	54.00 ± 10.35	62.00 ± 10.09

Although *R. decollata* is recognized as a non-specific biological control agent, our data demonstrate that, if given a choice, it will more likely select a plant food item over snail prey. Furthermore, *R. decollata* appears to only successfully kill snails with a shell diameter of <13mm and, as a result, sexually mature *C. aspersum* will likely escape predation in nature and continue to reproduce. Although the progeny of these snails will be prone to low rates of predation, many will likely develop to maturity. In fact, our bioassays demonstrated that when *R. decollata* was provided with snails within its preferred size range, predation occurred in <60% of bioassays. These data may help, in part, to explain the failure of *R. decollata* to reduce *C. aspersum* populations in southern California (Fisher and Orth, 1985).

Egg consumption by adult *R. decollata*, averaged 3.10 (± 0.50) eggs within a 7 day period. However, given that individual egg clutches of *C. aspersum* can contain > 150 eggs (Lazaridou-Dimitriadou *et al.*, 1998) and a single individual can lay >695 eggs throughout its life (Fisher and Orth, 1985), a large number of eggs will likely escape *R. decollata* predation in the field. Furthermore, gravid *C. aspersum* lay their eggs 25-40 mm below the soil surface (Dekle and Fasulo, 2001) and although *R. decollata* can burrow (Batts, 1957), the frequency with which it encounters subterranean *C. aspersum* egg nests is unknown. Therefore, unless the population of *R. decollata* is overwhelmingly large or predation efficacy is significantly higher in the field than indicated by laboratory bioassays, this ovicidal pressure is unlikely to be a significant driver of population decline in *C. aspersum*.

The results presented here questions strongly the widely held assumption that *R. decollata* is an effective predator of *C. aspersum* in California. There is an urgent need for rigorous manipulative field experiments to assess potential population level impacts that *R. decollata* could have on *C. aspersum*, especially in citrus.

The data presented in this article are adapted from Mc Donnell *et al.* (2016).

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Poster 3: Genetic Diversity and Origins of *Halyomorpha halys* in the U.S. and of Its Potential Biocontrol Agent Unexpectedly Recovered from the Wild in the United States

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The brown marmorated stink bug (BMSB), *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), is a highly polyphagous pentatomid that is native to Eastern Asia (China, Japan, Korea and Taiwan), (Hoebeke and Carter, 2003). Its pest status stems from feeding damage caused on a wide range of vegetable crops, fruit trees, and ornamentals. The first documentation of this pest in eastern North America occurred in Pennsylvania in 1996, and in western North America in 2004 in Oregon. It now has been detected in 43 states and is reported as a nuisance or agricultural pest in at least 26 states (Northeastern IPM Center, 2017). Over the last few decades, methods to reconstruct the invasion history of biological invasions (Estoup and Guillemaud, 2010), and in particular of human mediated invasions (Lombaert *et al.*, 2011), using molecular markers have improved substantially (Cristescu, 2015).

Using the cytochrome oxidase 2 and a fragment starting on the 12S ribosomal RNA and spanning part of the control region, Xu *et al.* (2013) detected only two haplotypes in BMSB collected in the northeastern U.S. From this, the authors concluded that BMSB might be introduced from the Bejing area in China. In the present study, we investigated the geographic origins of BMSB in the western U.S. (California, Oregon and Washington states) following the same approach as Xu *et al.* (2013). In total, 125 individuals were collected from the field, preserved in ethanol and shipped to EBCL for DNA extraction and subsequent genetic analysis. We detected a total of five haplotypes, two of them being already found in the eastern U.S. by Xu *et al.* (2013). While the eastern U.S. pest populations match a single source in the Bejing region in China, the overall genetic structure observed in the western U.S. suggested a more complex invasion scenario, encompassing several introduction events from different sources in China, including the

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Beijing area and also potentially from Japan. Moreover, our results do not rule out the hypothesis that the invasive population in eastern U.S. might have spread secondarily to the western USA.

As BMSB continues to spread to new areas in the region and populations increase, proactive research in these states has joined national efforts led by the U.S. Department of Agriculture and academic research institutions to develop a classical biological control program for BMSB under which a host specific natural enemy from the pest's native range is released in the pest introduced range (Lara *et al.*, 2016).

As a result, reconstructing the phylogeography of BMSB also provides a straightforward starting point for reconnaissance work on naturally occurring, host specific natural enemies in Asia. One of them, the egg parasitoid *Trissolcus japonicus* (Ashmead), (Hymenoptera, Scelionidae) has been under study in U.S. quarantine facilities since 2007 to evaluate its efficacy as a candidate classical biological control agent. Domestic surveys of egg parasitoids using sentinel egg masses of *H. halys* revealed that *T. japonicus* was already present in the wild in 2014 in Beltsville, Maryland, USA (Talamas *et al.*, 2015).

At the onset of the biological control program we developed 24 polymorphic microsatellites in order to assess the genetic diversity, including inbreeding level, of the quarantine colonies of the wasp. Taking advantage of this, we used these microsatellites in the present study to test the hypothesis that the populations recovered in Beltsville escaped from one of the eastern US quarantine facilities. Specimens recovered near Beltsville were preserved in ethanol and shipped to EBCL for DNA extraction. Given the haplodiploid nature of the wasp, only females were genotyped by three multiplexes of 8 loci each. Genotype analysis determined that the specimens collected in Beltsville do not match those from the US quarantine colonies (Fig. P3.1). The genotype analysis presented in this study is expanding to include populations of the wasp recovered in 2015 in Vancouver, Washington and 2016 in Marlboro, New York and Mercer, New Jersey1, and used for a more comprehensive analysis to pinpoint the origin of all recovered wasp populations.

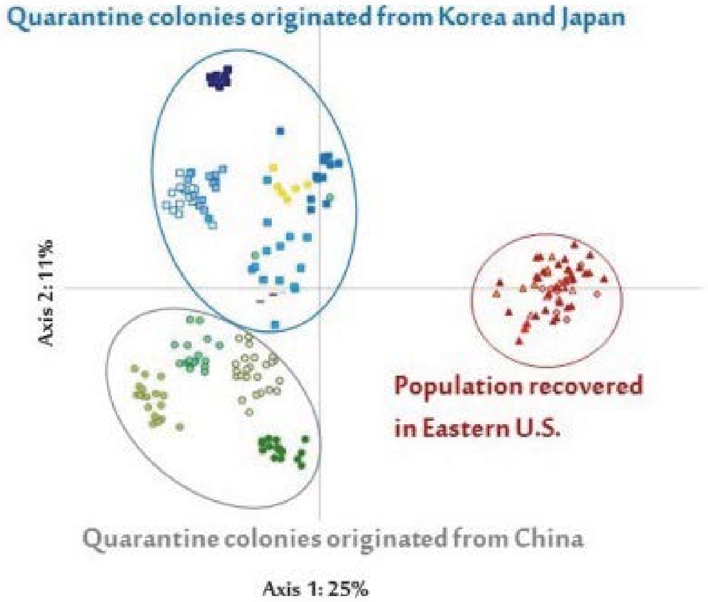

Fig. P3.1. Principal Coordinate Analysis (PCoA) based on 24 microsatellite loci of specimens of *Trissolcus japonicus* recovered in Beltsville, MD in Eastern U.S. and of all U.S. quarantine colonies originating from China, Japan and Korea. The results show that the field-collected parasitoids did not originate from the U.S. quarantine colonies.

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Poster 4: Variable Performance and Improvement by Crossing in Commercial Populations of the Pirate Bug *Orius majusculus*

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Populations of insect predators used in biological control may have different origins and have typically been maintained under laboratory conditions for many generations. They are therefore likely to differ genetically and may be genetically depauperate. Commercial arthropod predator populations used for biological control are therefore likely to differ in quality and control efficiency when applied in the greenhouse or in the field. However, genetic quality may be restored by crossing depauperate populations in a process known as heterosis, where the combination of complementary genes from both populations produce an offspring population that is of higher quality than the parental populations.

Pirate bugs are widely used in biological control against several arthropod pest species in greenhouses and agriculture. We tested three commercial populations of the pirate bug, *Orius majusculus* (Reuter) (Hemiptera: Anthocoridae), and their three possible crosses of two populations, for a number of performance characteristics. These included predation rate, starvation tolerance, basal activity, tolerance to heat and cold exposure, as well as body size and mass, and lipid storage. We then compared the various performance parameters between populations and analyzed whether crossing populations had resulted in heterosis.

The results showed a lower general performance in one of the three commercial populations. Furthermore, the F1 individuals obtained from the crosses on average had higher general performance, suggesting heterosis. However, the heterosis effects were inconsistent across performance parameters and crosses. We also found higher general performance of females than of males across parameters. One of the three commercial populations appeared to benefit from crossing with the other populations, whereas the other two populations were not affected by crossing.

The study highlights that commercial populations may differ in genetic quality and that crossing populations may be a useful tool for increasing performance of predators used in biological control. However, the effects observed in the current study were subtle, and both positive and negative effects of crossing populations were observed. Overall, one of the

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commercial populations appeared less fit than the other two and crossing this population with one of the other two populations appeared to improve population performance. We conclude that crossing populations improves fitness in these populations of *O. majusculus*, but only in the case where one population is less fit and benefits from the introduction of genes from outside. Therefore, it may be an advantage to introduce genes from a strong population to increase performance of some commercial populations.

Poster 5: Parasitoids of *Drosophila* in Switzerland and Their Potential for Biological Control of the Invasive *Drosophila suzukii*

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The spotted wing drosophila, *Drosophila suzukii* (Matsumara) (Diptera: Drosophilidae), is native to Asia and has recently achieved an almost worldwide invasion (Asplen *et al*., 2015). It lays its eggs into ripening undamaged fruits thereby causing large economic damage in stone fruit, berry, and vine cultivation (Mazzi *et al*., 2017). Control of *D. suzukii* remains challenging, as numerous overlapping generations are present infesting crop and non-crop fruits in various habitats. Hymenopteran parasitoids could be an element of a sustainable control strategy and have therefore received increased attention recently (Gabarra *et al*., 2015; Miller *et al*., 2015; Rossi Stacconi *et al*., 2015; Mazzetto *et al*., 2016).

We conducted a field survey in four regions of Switzerland to assess the presence of *Drosophila* parasitoids (Knoll *et al.,* 2017). Fruits infested with sentinel *Drosophila melanogaster* Meigen hosts were deployed in each of six fruit orchards and six semi-natural habitats (forest-patches or hedgerows) early, middle and late during the fruit-growing season. After four days of field exposure, samples were recollected and stored in a climate chamber. Emerging parasitoids were recorded and used to build up laboratory rearing. To investigate the parasitoids' ability to use *D. suzukii* as hosts, larvae and pupae of *D. suzukii* and *D. melanogaster* were offered in laboratory no-choice parasitization assays.

We collected eight hymenopteran parasitoid species (Table P5.1). The most common one was *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae), a generalist parasitoid of flies that even can act as a hyperparasitoid (Peters, 2009). Unlike *P. vindemmiae*, some parasitoids were only present in certain regions, such as *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae) that was found only in the southern part of Switzerland and *Asobara tabida* (Nees) (Hymenoptera: Braconidae) that was found only in the northern part.

Phenology varied among parasitoids: *A. tabida* and *Leptopilina heterotoma* (Thomson) (Hymenoptera: Figitidae) were captured in decreasing number from early to late season, while *L. boulardi* (Barbotin, Carton & Kelner-Pillault), *P. vindemmiae*, and *T. drosophilae* were captured in highest numbers during mid-season. To reduce the build-up of *D. suzukii* populations in spring, measures to enhance parasitoid numbers at that time could be advantageous.

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Table P5.1. Region, number of emerged parasitoids and number of traps with parasitoids from field collections 2014 und 2015. ZH: Zürich; TI: Tessin; TG: Thurgau; BL: Basel-Land. Adapted from Knoll *et al.* (2017).

Family, species	**Region**	**Number of Individuals**	**Number of Traps**
Braconidae			
Asobara tabida	ZH, TG, BL	58	9
Diapriidae			
Trichopria drosophilae	TI	520	9
Trichopria modesta	TG	4	1
Figitidae			
Leptopilina boulardi	ZH, TI, BL	2498*	39*
Leptopilina heterotoma	ZH, TI, BL, TG	695*	36*
Pteromalidae			
Pachycrepoideus vindemmiae	ZH, TI, BL, TG	7585	82
Spalangia erythromera	BL	62	2
Vrestovia fidenas	BL, TG	13	2

*2014: A total of 1836 *Leptopilina* sp. emerged from 17 traps.

The sole species that was more abundant in the crop habitats was *P. vindemmiae*, whereas *T. drosophilae*, *A. tabida* and *L. heterotoma* were predominantly found in the semi-natural habitats. Likely, these species are sensitive to temperature and/or humidity changes and therefore prefer semi-natural habitats, which provide rather stable conditions compared to the orchards. Semi-natural habitats could therefore play an important role to sustain natural enemies of drosophilids.

None of the larval parasitoids were able to reproduce on *D. suzukii* in the parasitization assay, although both *Leptopilina* species reduced the number of emerging *D. suzukii* significantly. In contrast, all pupal parasitoids used *D. suzukii* as a host and offspring numbers were comparable to those on *D. melanogaster*.

Thus, native parasitoids could contribute to the control of *D. suzu*kii and information on their phenology and habitat preference is particularly important in this context.

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Poster 6: Native North American vs. Asian Parasitoid Natural Enemies of Invasive Brown Marmorated Stink Bug

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The invasion of brown marmorated stink bug (BMSB), *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), in North America and Europe spurred efforts to characterize indigenous natural enemies attacking this pest in the native range and in invaded regions. At the time of the North American invasion, available literature on Asian natural enemies of BMSB was sparse, summarized by Qui (2008, 2010), and Lee *et al.* (2013). As interest in Asian natural enemies has grown for a possible biological control solution, recent surveys have compiled additional records from the native Asian range, particularly for egg parasitoids. Several species of *Trissolcus* egg parasitoids were found in most surveys. Recent taxonomic studies on *Trissolcus* of the Nearctic and Palearctic (Talamas *et al.* (2013, 2015a, 2017) will help make this task easier. The most widespread and important natural enemy in Asia appears to be the egg parasitoid *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) (also in literature as *T. halyomorphae* Yang), with high rates of parasitism reported in several studies (Qui, 2008, 2010; Yang *et al.*, 2009; Zhang *et al.*, 2017). Besides BMSB, *T. japonicus* also attacks several other species of Asian pentatomids (Yang *et al.*, 2009; Zhang *et al.*, 2015; Matsuo *et al.*, 2016; Kim *et al.*, 2017; Zhang *et al.*, 2017).

Abram *et al.* (2017) reviewed survey datasets from North America and Europe that comprise a variety of sampling methods, habitats, and geographic areas to consolidate available information and identify gaps in research. To date, the majority of research has focused on predators and parasitoids of *H. halys* eggs, using both sentinel and wild egg masses to characterize composition and impact of enemy communities (Fig. P6.1). Parasitism and predation rates are typically <10%, although they may be substantial in certain habitats, such as ornamental plant nurseries (Jones *et al.*, 2017). As yet, there is no evidence that resident North American parasitoids are adapting to BMSB (Dieckhoff *et al.*, 2017). This contrasts with the impact of egg parasitism in the native Asian range, which is considerably greater, suggesting the need for classical biocontrol to help manage this pest. Studies of natural enemies attacking *H. halys* nymphs and adults are less common to date but researchers are starting to focus more attention on resident predators and pathogens.

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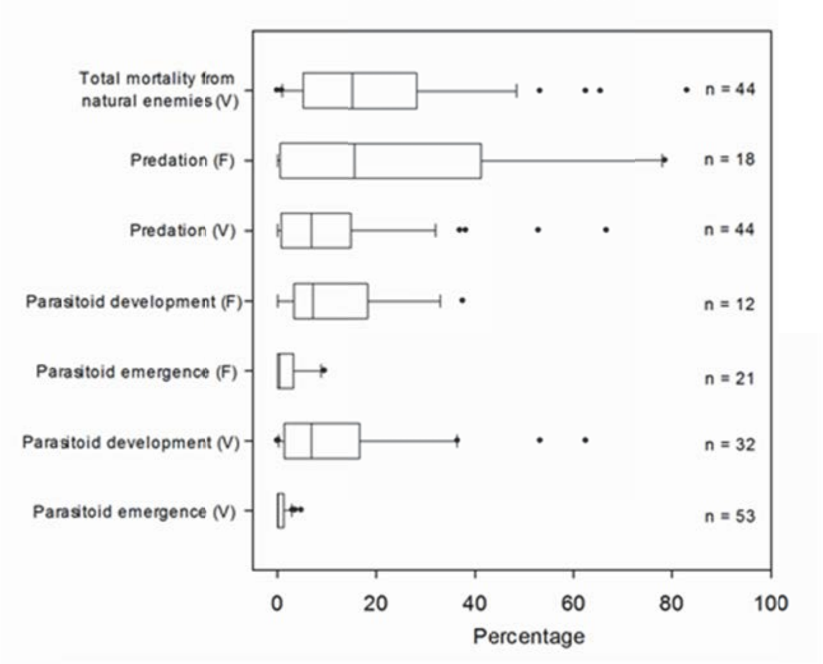

Fig. P6.1. Parasitism and predation of *H. halys* eggs (pooled North American and European surveys (from Abram *et al.* 2017). n= surveys, medians (vertical lines), 25th-75th percentiles (boxes), upper and lower deciles (whiskers), and outliers (points) for viable eggs (V, wild and sentinels pooled) and frozen sentinel eggs (F). Parasitoid development includes parasitism detected by dissections and emerged offspring. Total mortality includes surveys measuring both parasitism and predation.

U.S. researchers received an unexpected surprise when specimens of *T. japonicus* were identified in field survey samples collected near Washington D.C. during the 2014 field season (Talamas *et al.*, 2015b; Milnes *et al.*, 2016). This species was under study in quarantine laboratories but had not been released. Subsequent surveys have shown that the adventive population has established and is spreading, and that two additional adventive populations have been discovered in northeastern states and northwestern states. This will afford the opportunity to document the impact of this parasitoid on BMSB and non-target species in an invaded region.

Given the extensive crop damage that has occurred following the establishment of BMSB in North America, there is considerable concern that BMSB will continue to spread to other parts of the world. Using CLIMEX software, Kriticos *et al.* (2017) modelled BMSB's potential world distribution and reported that further spread in North America and Europe is possible, and that substantial parts of Africa, South America and Australasia are at risk. A more complete knowledge and understanding of the known BMSB natural enemies will help researchers to develop biological control strategies for newly-invaded areas.

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Poster 7: Insect Natural Enemies: Review and Future Application for CPB Management in Cocoa

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Insect natural enemies of cocoa pod borer (CPB), *Conopomorpha cramerella* (Snellen) (Lepidoptera: Gracillariidae), have been described and reported since the 1980's (Ooi, 1987). The study reported that these natural enemies attacked the eggs, emerging larva, pupa and the adult stage. A total of 24 different parasitoids have been reported attacking CPB in the south and southeast Asia region. Until now, only the egg parasitoids *Trichogrammatoidea bactrae fumata* Nagaraja and *T. cojuangcoi* Nagaraja (Hymenoptera: Trichogrammatidae) have been successfully mass produced and released into the field (Lim and Chong, 1987; Alias *et al.*, 2005).

Biological control using parasitoids that attack emerging larva and the pupal stage of CPB has never been reported. It was reported that natural parasitism was detected at significant levels only at the peak-crop period when CPB numbers are highest (Ooi, 1987). Success of predators, such as the cocoa black ants *Dolichoderus* spp. and *Anoplolepis* spp. (Hymenoptera: Formicidae), was limited only to certain localities. The availability of local natural enemies could help to reduce CPB populations below the economic threshold if the cocoa farm ecosystem able to support the existence of these natural enemies.

Natural enemies need shelter, alternate hosts and food sources for the adults (Landis *et al.*, 2000). In the case of CPB, biodiversity in the cocoa farm ecosystem should be encouraged. This could be achieved through reengineering the cocoa ecosystem landscape by adopting the concepts of landscape agriculture systems. A complex maize field landscape was reported to have resulted a significantly higher mean percentage parasitism of armyworm larvae compared to the simple landscape (Marino and Landis, 1996). Using this concept, the curent cocoa planting system should be revised (Fig. P7.1); other beneficial crop and flowering plants or patches of weeds need to be incooperated into the farm (Fig. P7.2). In this new cocoa planting system, biodiversity is encouraged and is expected to sustain the natural enemy populations and eventually will reduce CPB populations below the economic threshold.

The idea for revising the cocoa planting ecosystem is still an hypothesis. It is, however, a success story in other crop systems (Altieri, 2005). The number of natural enemies were increased and the damage due to insect pests remained below the economic threshold. An experimental plot is currently set up and crop loss due to CPB and the buildup of various natural enemies will be evaluated at that particular cocoa farm.

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Fig. P7.1. Current cocoa planting system that may not be able to sustain natural enemy survivability.

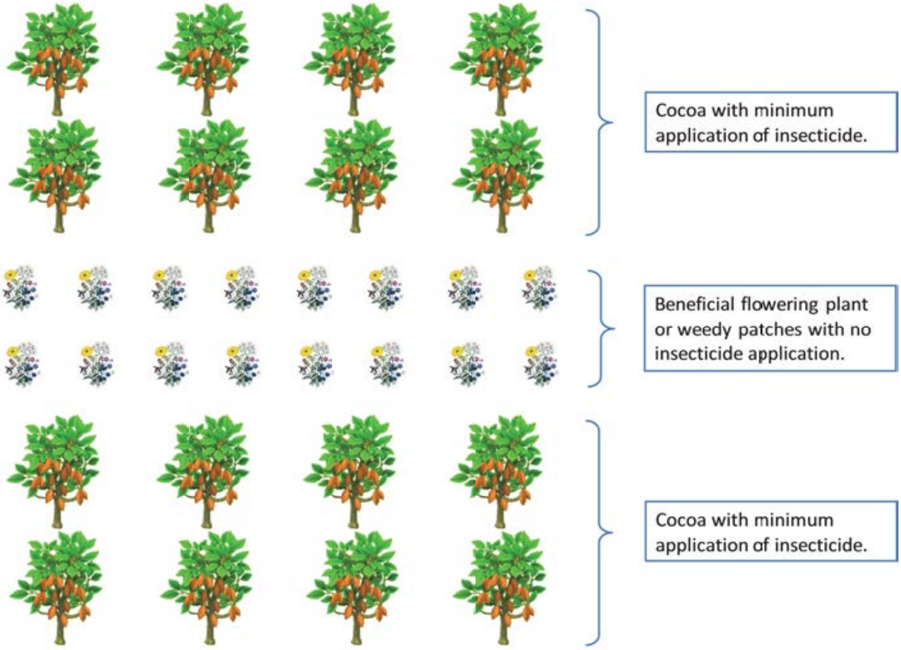

Fig. P7.2. Alternative cocoa planting system that encourages biodiversity for sustaining insect natural enemies in a cocoa farm.

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Poster 8: Do Invasive Alien Species Affect Diversity of Local Communities?

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Although the phenomenon of species extending their range is well known, intensive research on this topic only started in the late 1980s (Lockwood *et al.*, 2007). Particularly important are invasive alien species that have a demonstrable ecological or economic impact (Snyder and Evans, 2006; Lockwood *et al.*, 2007). It is sometimes suggested that a new species invading a new area causes a decrease in diversity of the community already present there, but this has rarely been satisfactorily proven by empirical data (Bahlai *et al.*, 2015). Preventing this decrease in diversity of native communities is a leading concern of conservation biology, which stimulated much research, dealing mainly with biological problems of species interactions. The empirical data for a direct test of such a decrease in diversity of native communities are missing in most cases, and very difficult to collect. Therefore, first some theoretical reasoning on the processes of temporal changes in dominance between species is useful. Comparison of temporal changes in abundance of species in a new community including alien species with those of the original community predicted by mathematical models may conceptually clarify our understanding of the effect of alien species.

Here we present results of simulation models to investigate the diversity dynamics in a theoretical system consisting of an "original" community of native species and an invasive alien species that invades this community, thus creating a "new" community. See Kindlmann *et al.* (2017) for full details of the simulations and results.

A series of virtual native communities living in spatially-explicit landscapes was created by means of simulations. We assumed an $\boldsymbol{S} = 24$ "native species" and a "community" (20×20 matrix) of "plants". Each element of the matrix was supposed to be inhabited by only one species. It was assumed that during the simulation either this species will survive or will be replaced by the invader. In the model this was represented by a 20×20 matrix $\boldsymbol{M} = (n_{ij})_{i,j=1,2,\ldots 20}$. A series of 400 "native populations", $\boldsymbol{P} = (p_1, p_2, \ldots p_{400})$, inhabiting the matrix was then created so that their species-abundance distribution followed a negative exponential. The "populations" $\boldsymbol{P}$ were then placed into the matrix $\boldsymbol{M}$ using two scenarios:

(a) "Random", when elements of $\boldsymbol{P}$ were placed in $\boldsymbol{M}$ successively and each element of P was placed into a vacant element of $\boldsymbol{M}$ completely at random.

(b) "Aggregated", when elements of $\boldsymbol{P}$ were placed in $\boldsymbol{M}$ successively by increasing species number, so starting from the commonest to the rarest species, starting from the right bottom corner of the matrix ($n_{20,1}$) and continuing to the left top corner ($n_{1,20}$).

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Five scenarios of invasion were considered. All elements of matrix ***M*** were occupied by native populations, with the exception of scenario (1), and all elements were distributed at random in the matrix, with the exception of scenario (5):

(1) Some cells in the matrix were initially unoccupied (thus there were less than 400 native populations distributed at random) and the invader randomly occupied some of the empty cells. Random choice of the cells invaded by the alien species was achieved by repeated generating of random pairs of numbers *i, j* from U(1,M), until an empty cell n_{ij} was found. That is, no competition occurred, the invader just occupied empty niches as not all elements of matrix ***M*** were occupied by native species.

(2) Cells occupied by the dominant native species were invaded and the native species replaced by the "strong" invader (an invasive alien species, which after its introduction first exterminates the dominant, most abundant native species). Random choice of the cells invaded by the invader was achieved by repeated generating of random couples of numbers *i, j* from U(1,M), until a cell occupied by the dominant species (species 1) n_{ij} was found.

(3) Cells occupied by the rarest native species were invaded, native species was replaced by the "weak" invader (a species that after introduction first exterminates rare native species). Random choice of the cells invaded by the invader was achieved by repeated generating of random couples of numbers *i, j* from U(1,M), until a cell n_{ij} occupied by the rarest species (species 24, 23, etc.) was found.

(4) Invaded cells were chosen at random within the whole matrix ***M***, independently of the native species present and the native species was replaced by the invader.

(5) Initial distribution of the native species was aggregated. Invaded cell was chosen at random within the whole matrix ***M***, independently of the presence of the native species and native species were replaced by the invader. Thus not all elements of matrix ***M*** (all native populations) were distributed at random in the matrix.

We calculated the following diversity measures for each of the communities considered:

(i) number of native species surviving to a particular stage of invasion,
(ii) Shannon-Wiener index *H* of the "new community",
(iii) Shannon-Wiener index *H* of the species surviving from the "original community".

Our results show that diversity changes over time during an invasion because of the increasing dominance of the invasive alien species, but the effect on the species already present may vary. Despite a large increase in numbers of the new invasive alien species, the diversity in the original community may be little affected (Gardiner *et al.*, 2014). The extent of the effect depends on several factors, including presence of free space (niches available for invasive alien species, which are not occupied by native species distribution of species in the original community, and types of interaction between alien and native species. As in scenario (1), adding an invasive alien species to an established community of "native" species initially positively affects the diversity in the community. This increase in diversity slows down as the invasive alien species spreads. Diversity then starts to decrease and finally converges to zero, when the invasive alien species replaces all native species. A corollary is that the diversity in the new community may decline with little or no parallel change in the diversity in the original community of native species. This is very important in cases when native species become rare before the arrival of an invasive alien species and leave "free space" for its spread, because other drivers of population change may play a role, like habitat deterioration or climate change (Dixon and Honek, 2014; Bahlai *et al.*, 2013).

The process of colonization of an area by invasive alien species affects the diversity in the community. The magnitude of this effect depends on the proportion of the available "space" that the invasive alien species occupies. Our simulations revealed a particularly interesting situation that occurs when native species are replaced randomly. In this case the diversity in the "original" community for a long time did not reflect the increase in abundance of invasive alien species, except at the final stage, when nearly all cells are occupied by the invasive alien species. This conservative behaviour may call for cautious interpretation of the small changes in species diversity observed in native communities after the invasion by *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidaae) in Central Europe (Honek *et al.*, 2013). Minute differences may conceal an advanced stage of the *H. axyridis* invasion that might have negative consequences for the "original" community in future.

Our study conceptually clarifies several situations, which differ in important characteristics: availability of free space within the original community, competitive relationship between the invasive alien and native species and distribution of native species, without entering into details of biology. Further, this study investigates separately the diversity in the "original community", i.e., the community of native species, which survive during the process of invasion of an invasive alien species, and diversity in the "new community", which includes native species and invasive alien species. This distinction has interesting implications, because the trajectories of the change in diversity in the "original" and "new" community substantially differ in many situations.

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Poster 9: Introduction to FAO Guide: Classical Biological Control of Insect Pests in Forestry: A Practical Guide

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An increase in travel, trade volumes and speed of transport have facilitated the introduction of non-indigenous insect species in new environments. Many of these species have become invasive within their new environments and have caused substantial damage by threatening the ability of forests to provide their economic, environmental and social benefits. Climate change also appears to be compounding the problem by increasing the probability of invasive forest pest establishment as well as providing conditions that allow some species to become more serious pests in their native range.

The Integrated pest management approach exists for managing pests and includes deployment of resistant or tolerant germplasm, chemical control, silvicultural management, and biological control. Biological control is a three-prong approach widely used in planted and natural forests; conservation biological control – where the host environment is manipulated to maximise the effectiveness of existing natural enemies; augmentation biological control - where natural enemies are mass-produced and released against a pest; and classical biological control – where natural enemies are imported from the endemic range of an introduced pest and released in the new environment. Classical biological control is particularly suited as a management tool for invasive forest pests that invade new environments, and can provide efficacious, environmentally friendly, long-term control of these pests with minimal ongoing costs.

Examples of successful classical biological of forest insect pests include the Sirex woodwasp, *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) in pine plantations in the southern hemisphere (Hurley *et al.*, 2012), cypress aphid, *Cinara cupressi* (Buckton) (Hemiptera: Aphididae) on cypress in East Africa (Day *et al.*, 2003), eucalypt gall wasps, *Leptocybe invasa* Fisher & LaSalle and *Ophelimus maskelli* Ashmead (Hymenoptera: Eulophidae) in Israel (Kim *et al.*, 2008; Protasov *et al.*, 2007), *Eucalyptus* snout beetle, *Gonipterus platensis* Marelli (Coleoptera: Curculionidae), one of the first successful cases in eucalyptus plantations and forest plantations in general (Rivera *et al.*, 1999). Other biological control efforts in forests are currently underway, including against the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) (Bauer *et al.*, 2008), and

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bronze bug, (*Thaumastocoris peregrinus* Carpintero & Dellapé, (Hemiptera: Thaumastocoridae) (Mutitu *et al.*, 2013).

While biological control is an excellent management tool for many pests; endemic and exotic, the process of developing and implementing a biocontrol program can be complex. At present, no general guidelines exist for the classical biological control of forest insect pests, particularly in a format that would benefit developing countries that do not have easy access to this information in their own language.

To address this gap, the Food and Agriculture Organization of the United Nations (FAO) is leading a multistakeholder process to develop a practical guide, *Classical biological control of insect pests in forestry*. The guide will be based on best practice principles using existing publications (e.g., International Standard for Phytosanitary Measures 3) and will detail the 'why' (background and understanding) as well as the 'how' (procedures) on controlling forest pests using classical biological control.

This Guide will provide information from both a theoretical and practical point of view that will allow forest pest managers worldwide to gain the practical understanding they need to implement successful classical biological control programs against the key forest pests within their countries

Minimizing the impacts of pests on forest resources through improved pest management will allow countries to better access the economic, environmental and social benefits that planted and native forests provide.

By providing a detailed framework on which biological control relevant to local needs can be implemented, the guide for *Classical Biological Control of Insect Pests in Forestry* will benefit, policy makers, forest management decision-makers, educators and forest health practitioners who implement pest management activities at the ground level. It will support sustainable forest management by, where appropriate, assisting in reducing reliance on other less environmentally friendly pest control methods such as chemical insecticides.

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Poster 10: Diversity of Edible Saturniids (Lepidoptera: Saturniidae) and Their Parasitoids in Kenya

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Over 2,000 edible insect species are consumed globally by about 2 billion people (van Huis, 2013). Consumption of edible insects has been strongly recommended as a strategy to enhance food and nutritional security in Africa (Kelemu *et al.*, 2015), where approximately one in four people are estimated to be undernourished. Caterpillars, especially those belonging to Saturniidae are a key group of edible insects in the central and southern African regions (Kelemu *et al.*, 2015). Nutritionally, the saturniids are rich in proteins which often constitute more than 50% of their body weight (Omotoso, 2016). The most widely consumed saturniids in Africa are Mopane worm, *Gonimbrasia belina* (Westwood) in southern Africa; Cabbage tree emperor moth, *Bunaea alcinoë* (Stoll) in West and Central Africa and the pallid emperor moth or Shea butter caterpillar, *Cirina forda* (Westwood) in west, central and southern Africa. Despite their high diversity in eastern Africa (Kioko, 2011), consumption of these saturniids is not widespread in that region, and it has been reported only from coastal Kenya. Efforts to document the diversity of the Saturniidae and their natural regulatory factors are minimal and this was the primary focus of this study.

Methods: Roving surveys were undertaken in different agroecosystems in Kenya namely, the coast region, eastern lowlands, central highlands and the semi-humid lake zone in the west. Various saturniid caterpillars were collected and reared to adulthood in the laboratory. Identification of the saturniids were undertaken both morphologically based on the adult moth characteristics (Pinhey, 1956) and at molecular level through barcoding using Lep (Hebert *et al.*, 2004) and LCO1490/HCO2198 primers (Folmer *et al.*, 1994).

Results: A high level of saturniid diversity was observed across Kenya, with distinct ecological and host preferences (Table P10.1 and Fig. P10.1).

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Table P10.1. Diversity of edible Saturniids (Lepidoptera: Saturniidae) in Kenya.

Name	Common name	Host plants	Regions of distribution
African emperor moth	*Gonimbrasia zambesina* (Walker)	*Mangifera indica* L. (Mango)	Coast and semi-humid lake zone in the west
Cabbage tree emperor moth	*Bunaea alcinoe* (Stoll)	*Balanites aegyptiaca* (L.) Delile (Desert date)	Coast, eastern lowlands and semi-humid lake zone in the west
Pine tree emperor moth	*Nudaurelia krucki* Hering	*Schinus molle* L. (California pepper tree)	Central highlands
Mopane worm	*Gonimbrasia belina* (Westwood)	*Acacia* sp.	Eastern lowlands
Pallid emperor moth	*Cirina forda* (Westwood)	*Vitellaria paradoxa* C. F. Gaertn. (the African Shea tree)	Semi-Humid lake zone in the west
Speckled emperor	*Gynansia maja* (Klug)	*Acacia* sp.	Eastern lowlands

Fig. P10.1. Mature larvae of different saturniids in Kenya. **a)** *Gonimbrasia belina*; **b)** *Bunaea alcinoe*; **c)** *Nudaurelia krucki*; **d)** *Gonimbrasia zambesina*; **e)** *Cirina forda*; **f)** *Gynanisa maja*.

Other saturniids infrequently observed were *Holocerina angulata* Aurivillius in the central highlands and the chestnut emperor, *Melanocera menippe* Westwood, from the coastal region. Molecular characterisation of the red and black coloured forms of *B. alcinoe* from west and east Africa, respectively, revealed that the two colour forms were not similar and most likely different species with more than 20% genetic distance.

Natural enemies of Saturniids in Kenya: Larval-pupal parasitic dipterans belonging to the Tachinidae (Diptera) such as *Senometopia* sp. (cf. *evolans* Wiedemann) from *G. zambesina* and an apparently undescribed species of *Ceromyia* Robineau-Desvoidy from *B. alcinoe, G. belina* and *G. maja* were recorded. The large larval parasitoid *Euryophion pisinnus* Gauld & Mitchell (Hymenoptera: Ichneumonidae) was frequently encountered from *B. alcinoe*. The larval parasitoids, *Aleiodes trifasciatus* (Enderlein) (Hymenoptera: Braconidae) was observed to emerge from 2nd and 3rd instars of *G. zambesina*. Other braconids belonging to *Cotesia* sp. were also observed on *G. belina*, *G. zambesina* and *B. alcinoe*. Egg parasitoids of *N. krucki* belonging to the genus *Eupelmus* (Hymenoptera: Eupelmidae) were also key parasitoids observed for the first time on this species (Peigler, 1994).

Conclusion: The lack of widespread consumption of edible saturniids, despite their high diversity in the Kenya highlights the need for greater awareness on the economic potential and nutritional benefits of saturniid caterpillars as human food in east Africa. Furthermore, detailed understanding of the key natural enemies regulating saturniid populations in the wild will be critical to establish on-farm rearing protocols for these insects.

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Poster 11: Action of *Bacillus thuringiensis* on *Eucalyptus* Snout Beetle *Gonipterus platensis* (Coleoptera: Curculionidae) Larvae

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Wood production in tropical and subtropical regions is based on forest plantations, mainly with *Eucalyptus* spp. In Brazil eucalyptus occupies 72% of the total area of forest plantations (IBA, 2016). In last two decades, occurrence of invasive pests has affected the wood productivity in many countries. The main invasive insect pests to eucalyptus are *Glycapis brimblecombei* Moore (Hemiptera: Aphalaridae), *Thaumastocoris peregrinus* Carpintero and Dellapé (Hemiptera: Thaumastocoridae), *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) and *Gonipterus platensis* Marelli (Coleoptera: Curculionidae) (Schühli *et al.*, 2016).

The *Gonipterus* complex (Mapondera *et al.*, 2012) is the main group of coleopterous defoliators of eucalyptus, considering introductions into new areas and increasing outbreaks (Hurley, 2016), and reduction of biological control levels by *Anaphes nitens* Girault (Hymenoptera: Mymaridae) and by entomopathogenic fungi *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Clavicipitaceae) (Reis *et al.*, 2012). Nowadays the eucalyptus snout beetle *G. platensis* is the main species occurring in eucalyptus plantations in São Paulo and Parana states. Another biological option is *Bacillus thuringiensis* Berliner (Bacillaceae), already evaluated against other coleopterous defoliators in Australia and New Zealand (Elek and Beveridge, 1999; Whiters *et al.*, 2013).

In this study, 1st instar larvae of *G. platensis*, reared in laboratory, were exposed to isolates of *B. thuringiensis* var. *israelensis* (Bti), *B. thuringiensis* var. *tenebrionis* (Btt) and *B. thuringiensis* var. *kurstaki* (Btk) in different concentrations, to evaluate isolate efficiency to control this pest. The bioassays were carried out in controlled conditions (temperature: 25 ± 1°C, RH: 70 ± 10 % and photophase: 12 h). The *B. thuringiensis* isolates were cultivated in Petri dishes with Nutrient-Agar medium and incubated to 30ºC for seven days. The bacterial cultures were collected and diluted in autoclaved distilled water. Tween-20 at 0.05% was added to the suspensions and they were quantified in a Neubauer chamber to standardize the concentrations. Young leaves of *Eucalyptus urophylla* S. T. Blake (Myrtaceae) were cleaned and submerged in a suspension of spores/crystals of the respective isolates for 1 min and allowed to dry in a flow chamber for 15 min. After this the leaves were placed in plastic pots. Larvae of *G. platensis* were transferred to the leaf surface. The evaluation was made after 72 h. The experimental design was completely randomized with nine treatments and three replicates with 10 larvae in each one.

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Isolates of Bti and Btt showed insecticide activity on 1st instar larvae of *G. platensis*, with the Bti isolate more effective than the Btt isolate in this bioassay (Table P11.1). The Btk isolate (negative control) that expressed Cry1 e Cry2 proteins (Crickmore, 2016), the basic commercial formulations used to control lepidopterous pests in forestry in Brazil, was ineffective on *G. platensis*, confirming its specificity to Lepidoptera (Tabashnik, 2015). The Bti and Btt isolates have different compositions in their protein crystals. The Bti isolate expresses the proteins Cry4, Cry10, Cry11, Cry60, Cy1 and Cyt2 (Crickmore, 2016) and its pathogenicity has already been reported for another coleopterous pest, the cotton bollweevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) (Martins *et al.*, 2007). The Btt isolate expresses the proteins Cry3 and Cyt2 (Crickmore, 2016), normally used in bioinsecticide formulations to control Coleoptera in agriculture and forestry (Elek and Beveridge, 1999; Whiters *et. al.*, 2013; Kim *et al.*, 2015).

Table P11.1. Mortality of *Gonipterus platensis* larvae exposed to different concentrations of *Bacillus thuringiensis* isolates (Temp.: 25±1 °C, Rh: 70±10 %, photophase: 12 h).

Concentration (spores.crystals/mL)	Mortality (%)		
	Btk	Bti	Btt
1.0×10^{10}	0	100	100
3.0×10^{9}	-*	97	71
1.5×10^{9}	-	69	-
1.0×10^{9}	-	-	63
6.0×10^{8}	-	-	50
3.0×10^{8}	-	29	23
1.5×10^{8}	-	14	10
1.0×10^{8}	-	-	0
3.0×10^{8}	-	13	-

* no data

The pathogenicity of Bti and Btt isolates shows possibilities for eucalyptus snout beetle management, as new specific bioinsecticides. The study of the protein toxins of *B. thuringiensis* against *G. platensis* is important to select the best isolates to control larvae, the stage that causes more damage than adults, and opens the possibility to incorporate specific protein genes into eucalyptus trees, for plant resistance. Further studies to verify interactions among toxic proteins effective against *G. platensis* larval control are necessary.

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Poster 12: Host Specificity Testing of *Psyllaephagus bliteus*, an Accepted Biocontrol Agent of *Glycaspis brimblecombei*, Reveals a New Host

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The risk of releasing *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) in South Africa to control the lerp-forming eucalypt pest, *Glycaspis brimblecombei* (Hemiptera: Psyllidae), was evaluated using no-choice tests. The chosen non-target test insects included the only native lerp-forming psyllid in South Africa, as well as three non-native psyllids that occur on eucalypts, one of which, *Spondyliaspis* cf. *plicatuloides* (Froggatt) (Hemiptera: Psyllidae) forms lerps. *Psyllaephagus bliteus* did not show interest in three test species, but it did oviposit and develop in *S.* cf. *plicatuloides*. The risk to native species is thus low. The inclusion of non-native species on the same host as test species provided a better understanding of the host range of this agent.

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Poster 13: Potential of Entomopathogenic *Hyphomycetes* for Control of Forest and Urban Lepidoptera in Georgia

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Gypsy moth, *Lymantria dispar* L., Fall webworm, *Hyphantria cunea* Drury (Lepidoptera: Erebidae), and Mottled Umber, *Erannis defoliaria* (Clerck) (Lepidoptera: Geometridae), are polyphagous, univoltine pest species, that are destructive defoliators of a wide range of broad leaf trees of forests, orchards, fruit trees (Rosaceae), and shrubs. They are economically important forest, orchards and urban pests in Georgia, where successive defoliations over several years can cause growth loss, branch dieback and eventual tree mortality. To date, various strains of entomopathogenic fungi, such as *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Clavicipitaceae), *Lecanicillium* (previous name, *Verticillium*) sp. (Cordycipitaceae) and *Paecilomyces* (Trichocomaceae), have been used to control lepidopteran larva (Quesada *et al.*, 2006; Steenberg and Humber, 1999; Hassani, *et al.*, 2000; Shia and Feng, 2004; Malarvannan *et al.*, 2010; Han *et al.*, 2014)

The appropriate use of environment-friendly microbial pesticides can play a significant role in sustainable crop production by providing a stable pest management program. Among the fungi, several asexual species are associated with arthropods, especially insects. Entomopathogenic fungi that parasitize insects are valuable weapons for biocontrol and play an important role in promoting integrated pest management (Bruck *et al.*, 2005).

Recent investigations of entomopathogenic Hyphomycetes, that were isolated from different geographical locations and natural environments of Georgiastudied the virulence against *L. dispar*, *H. cunea* and *E. defoliaria* . Three isolates: *Beauveria bassiana*, *B. pseudobassiana* Rehner & Humber, *Metarhizium anisopliae* (Metchnikoff) Sorokin (Clavicipitaceae) were evaluated on larvae in the laboratory.

The 5th to 7th larval instars (L5-L7) of *L.dispar*, *H. cunea* and *E. defoliaria* were collected from natural sites in east and west Georgia. Local strains of Bb024 (IMI#50179), Bpsb 010 and *Metarhizium anisopliae* M7/2 (IMI#501805), were identified using molecular tools and deposited in the CABI-UK Genetic Recourse Collection.

To assess virulence, one concentration of 1.0 X 10^8 conidia ml^{-1}, of each fungal isolate was tested against each pest species and compared with control insects. Dead or infected larvae with fungal symptoms were removed and placed in a moist environment for development of conidia. Mortality of larvae was recorded 3-18 days after treatment. Results are given in Fig. P13.1.

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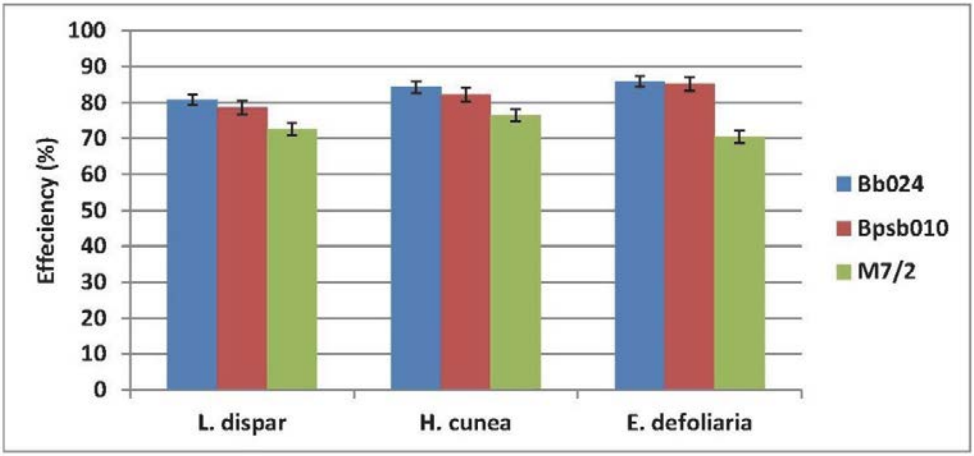

Fig. P13.1. Pathogenity of local isolates to larvae of *Lymantria dispar, Hypanthria cunea* and *Erania defoliaria.*

The maximum mean mortality of *L. dispar, H. cunea* and *E. defoliaria* larvae were noted at 18 days post-inoculation were 80.8% , 84.3% and 85.9% for Bb024; 78.7%, 82.3% and 85.3% for Bpsb010; and 72.6%, 76.5% and 70.5% for M7/2. All fungal strains were pathogenic to the targeted insect larvae. Maximum larvae mortality was observed 5-9 days after treatment, whereas in contrast Bpsb010 mortality occurred later f 5 -13 day. However, virulence varied considerably.

Development of mycosis was variable to Bb024 was observed in the L5-L6 and cocoon of all target insects. With *M. anizopliae* M7/2 symptoms of mycosis were mostly observed in L5-L7. In contrast, Bpsb010 mycosis symptoms were mostly observed in L6-L7, but rapid development of mycosis was observed in variance of Bb010. It should be noted that, in the case of Bb0024 dimorphic males of *H.cunea* emerged with undeveloped wings.

The results suggest that local isolates can be used to control lepidopteran pests of forest, orchard and urban trees.

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Poster 14: Diversity of Entomopathogenic fungi from forest ecosystem of Georgia

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The soil provides excellent habitats and reservoirs for diverse strains of insect pathogenic fungi and other microorganisms because it is buffered against extreme biotic and abiotic influences. Soil-inhabiting entomopathogenic fungi are an important and widespread component of most terrestrial ecosystems, they play a key role in regulating invertebrate pests and have a cosmopolitan distribution, with potential for use in biocontrol. (Keller and Zimmerman, 1989).

Many species belonging to the Hypocreales (Ascomycota) such as *Beauveria* spp., *Metarhizium anisopliae* (Metchnikoff) Sorokin and *Paecilomyces* spp. inhabit the soil for a significant part of their life cycle when they are outside of their insect host and are especially common. (Keller and Zimmerman, 1989; Jackson *et al.*, 2000; Domsch *et al.*, 1980).

The objectives of the present study were to explore diverse habitats as potential sources of local strains of entomopathogenic fungi. Specifically, this study aimed to explore the distribution of entomopathogenic fungi in soils from forest habitats of Georgia. Isolation of indigenous entomopathogenic fungi is essential to provide an insight into naturally occurring fungal biodiversity and to provide a pool of potential biological control agents to be conserved or inundatively released into the forest ecosystem for pest control.

During 2010-2016a total of 48 soil samples were collected from four different sites and at altitudes 600-1700 meter above sea level (masl), representing different forest habitats of east and west Georgia (Table P14.1). Samples were collected with a cylindrical probe to a depth of 25 cm after removal of surface litter and were placed in clear plastic bags for transport to the laboratory.The soil samples were sieved through a 2 mm mesh and stored at 4^oC for a few days.

Isolation of the entomopathogenic fungi was achieved using the ‘Galleria bait method’ (Zimmermann, 1986) modified for *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). Each soil sample was baited with 10 fifth instar larvae of *T. molitor* and the cups were incubated in the dark in closed cardboard boxes at room temperature of 25 ±3 °C. Samples were examined at 5 and 20 days. They were checked dailey to detect symptomatic or dead larvae, that were removed and placed individually in Petri dishes with a moist environment for conidia development. To isolate entomopathogenic fungi different artificial media, SDAY and PDA were used.

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Table P14.1. Sites sampled in Georgia for entomopathogenic fungi.

	Sites	Coordinates	Altitude (masl)	Habitat description	Region
1	Botanical garden	41°41'27.84"N/ 41°42'27.08"E	80	Forest	
2	Barjis khevi	41°40'22.81"N/45°50'5.17"E	583	Forest fir-grove	Adjara West Georgia
3	Mtirala	41°42'5.45"N/41°53'21.66"E	930	Forest	
4	Khala	41°42'31.82"N/41°47'49.37"E	154	Forest box-tree	
5	Botanical garden	41°41'27.84"N/41°42'27.08"E	45	magnolia	
6	Botanical garden	41°41'27.84"N/41°42'27.08"E	60	Forest	
7	Botanical garden	41°41'27.84"N/41°42'27.08"E	65	Forest fern zone	
8	Ilia lake	41°57'2.99"N/45°47'47.41"E	415	Forest	Kakheti
9	Lagodekhi	41°49'23"N/45°34'12"E	677	Forest	
10	Dusheti	42°04'33"N/44°43'51"E	40	Forest	
11	Ackuri	41°44'51"N/43°10'22"E	1200	Forest	Samtskhe Javakheti
12	Rabari	41°34'29"N/42°51'47"E	1400	Forest	
13	Patara tsemi	41°44'12"N/43°32'14"E	1600	Forest	
14	Bakuriani	41°45'45"N/43°30'58"E	1750	Forest	
15	Teleti	41°38'29."N/43°48'75"E	60	Forest	
16	Jinvali	42° 6'36.97"N/44°46'15.64"E	750	Deciduous Forest	
17	Khando	41°29'29.82"N/43°24'12.48"E	1645	Forest,fir-grove	Mtskheta Mtianeti
18	Gudauri	42°28'31.94"N/44°28'49.70"E	2197	Forest	
19	Gudauri	42°28'31.94"N/44°28'49.70"E	2197	Forest	
20	Gergeti	42°39'44.77"N/44°37'13.63"E	2183	Forest birch wood	
21	Jvari mountain	41°50'18.16"N/44°44'2.52"E	612	Pasture	
22	Kobi	41°47'9.13"N/44°49'5.48"E	476	Forest, near river	

Entomopathogenic fungi were isolated from 22 of the 48 (45.3 %) soil samples. The following taxa (% of sample) were found: *Beauveria bassiana* (Balsamo-Crivelli) Vuillimen (63.5 %), *Metarhizum* (15.8%), *Lecanicillium* sp. (7%), *Isaria fumosoroseus* Wize (5.5%), *Paecilomyces* sp. and *Fusarium* sp. (3.5 %) and *Entomopthora* sp. (1.75%). In these samples the most common species *Beauveria* spp and *Metarhizium* spp. were isolated, and no entomogenous fungi were isolated from 26 (56.7%) of the soil samples (Fig. P14.1).

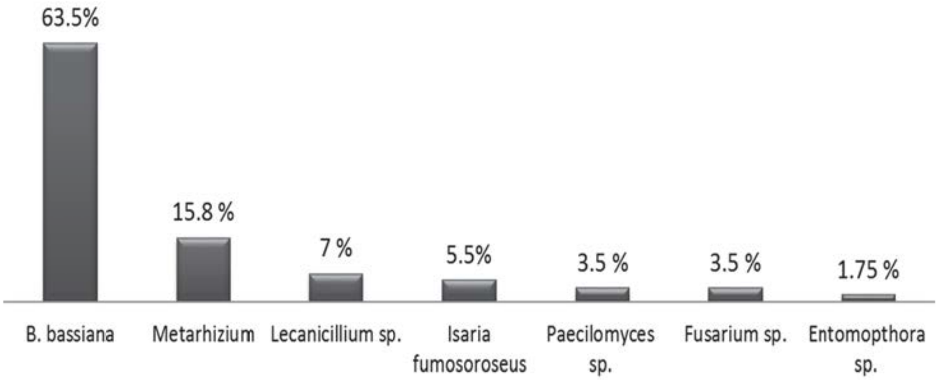

Fig. P14.1. Comparison number of EPF isolated from soil.

Investigations for selection of several local fungal species from pest or soils ecotypes from different environmental conditions and climate zone, are a most important subject, that local strains may be better adapted to local conditions and will be successfully used them as biological control agents in IPM strategies.

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Poster 15: Innate Positive Chemotaxis to Pollen from Crops and Banker Plants in Predaceous Biological Control Agents: Towards New Field Lures?

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Insect predators and parasitoids provide valuable ecosystem services as biological control agents of key pests that infest cultivated crops. However, natural enemies often need extra resources, besides those provided by the crop itself. These may include additional prey, additional food resources for adults (specifically nectar and pollen), and overwintering habitat and hiding places/shelter. Previous studies have indicated that releases of predatory or parasitic insects accompanied by the introduction of specific resource plants is one of the most effective ways for enhancing the effectiveness of biological control in agro-ecosystems. These plants are classified by their function in habitat management into categories such as honey plants (floral plants), banker plants, trap plants, etc., especially *Orius* spp. (Hemiptera: Anthocoridae). In the agricultural landscape, floral plant systems are designed to enhance the efficacy of biological control exhibited by natural enemies by providing an alternative source of food when prey is scarce or absent.

The flower bug *Orius sauteri* (Poppius) (Hemiptera: Anthocoridae) is widely used as a biocontrol agent against thrips and aphids infesting greenhouse vegetables in Asia. We investigated the effects of the plant *Calendula officinalis* L. (Asteraceae), used as a floral resources, for promoting the control of *Myzus persicae* (Sulzer) (Hemiptera: Aphidiae) and *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) by *O. sauteri* under laboratory and greenhouse conditions.

Results showed that the presence of *C. officinalis* enhanced aphid and thrips suppression via an increas in *O. sauteri* population growth. The predator populations responded positively to the addition of *C. officinalis* in the system and they varied also as a function of the temperatures tested under laboratory conditions. In a similar way, predator populations varied among seasons with the highest densities recorded in May in the greenhouse.

Calendula officinalis can be used to increase available resources for natural enemies used in agricultural crops, notably in greenhouses. This study also provides evidence that increasing floral resources can enhance pest suppression provided by *O. sauteri*.

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Poster 16: Estimating Parasitoid Suppression of Aphid Populations in the Field

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We developed and validated a quantitative method to assess the capacity of parasitoids to reduce peak aphid densities under field conditions. Our method was built upon an existing model describing the bell-shaped population dynamics observed in many aphid species and estimates the impact of early parasitoid-induced mortality on forthcoming aphid populations. We validated the model using the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), and its most abundant parasitoid in North America, *Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae). Soybean aphid populations were well described by the model, facilitating the prediction of peak aphid densities and its timing from the date of field colonization onward. The model estimated that *A. certus* reduced peak soybean aphid densities by only 1-6% in 2012 and 2013 because of low levels of parasitism early in the season. The method we propose is simple and could be extended to other natural enemies.

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Poster 17: Does Patch-guarding Behaviour in Parasitoids Deter or Attract Arthropod Egg Predators?

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Host patch defence has been reported in several parasitoid taxa. Typically, a patch-guarding parasitoid female finds a patch, oviposits and patrols the patch for some time. Patch guarding has been shown to reduce competition. We quantified a potential drawback of patch defence, i.e. increased predation on developing parasitoids, using *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) exploiting eggs of the brown marmorated stink bug, *Halymorpha halys* Stål (Hemiptera: Pentatomidae). We hypothesized that parasitoids either deter predators with aggressive behavior or attract them with their movement. In the laboratory, we recorded the behavior of *T. podisi* protecting their patch against *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae). Patch-guarding increased the residence time of predators on egg masses from 6% to 55% and the number of eggs consumed from 17% to 68%, depending on the predator species. In field experiments, predation rates were 50% higher on egg masses guarded by a parasitoid female.

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Poster 18: The Behavioural Type of a Top Predator Drives the Short-term Dynamic of Intraguild Predation

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Variation in behaviour among individual top predators (i.e., the behavioural type) can strongly shape pest suppression in intraguild predation (IGP) (Sih *et al.*, 2012). However, the effect of a top predator's behavioural type – namely, foraging aggressiveness (number of killed prey / time) and prey choosiness (preference degree for certain prey type) – on the dynamics of IGP may interact with the relative abundances of top predators, mesopredators, and pests. Here, we investigated such an interaction using a system consisting of three species: a top predator, the spider *Philodromus buchari* Kubcová (Araneae: Phildromidae) (henceforth, the top predator); a mesopredator, the spider *Dictyna* sp. (Araneae: Dictynidae) (henceforth, the mesopredator); and a pest, the psyllid *Cacopsylla pyri* (L.) (Hemiptera: Psyllidae) (henceforth, the pest). To investigate the dynamics we used a simulation model, with parameters estimated from laboratory experiments and field observations.

First, we investigated the relative preferences of the top predator for the mesopredator and the pest. After that, we measured the top predator's foraging aggressiveness as the number of pest individuals killed per hour. We then studied the functional responses of the top predator's behavioural types to the pest and the mesopredator. To obtain the parameters for the simulation model we also investigated the functional response of the mesopredator to the pest. Finally, we developed a model of the IGP system. The model aimed to simulate the dynamics of IGP during a 30 day period in April, because the main contribution of generalist predators as biocontrol agents is, to slow the population growth of the pest at the beginning of the season until the specialized predators arrive (Symondson *et al.*, 2002). Because the effect of the top predator can depend on the abundance ratios among the three species in the IGP system, we simulated the dynamic by maintaining high abundances of pests while varying the abundances of the mesopredator and the top predator. Detailed descriptions of the methods and the model can be found in Michalko and Pekár (2017).

We found that the timid individuals of the top predator spider *Philodromus buchari* kill fewer psyllid pest *Cacopsylla pyri* and are choosy, because they prefer the pest over the mesopredator spider *Dictyna* sp. In contrast, the aggressive individuals kill more pests and do not prefer pests over the mesopredator. Using a simulation model, we showed that the suppression efficiency of the behavioural types of the top predator is context-dependent. The aggressive/non-choosy individuals can be effective biocontrol agents in situations when the mesopredator is scarce in the environment or the top predator is highly abundant. The system is generally more effective with abundant mesopredators, but the aggressive

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individuals of the top predator decrease the number of mesopredators more rapidly. Thus, the system with abundant aggressive top predators and abundant mesopredators can be effective in univoltine agroecosystems (e.g., fields of grain), while in perennial agroecosystems (e.g., orchards) the system with timid/choosy top predator individuals might be more effective from the long-term point of view. From the short-term and probably also from the long-term point of view, timid/choosy top predators would also be profitable when the mesopredator is abundant and when the top predator achieves only moderate abundances. To improve biocontrol efficiency of predators, research on the effect of their behavioural types on ecological dynamics and research on the alteration of their behavioural types are needed.

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Poster 19: The Effect of Plant Resistance on Biological Control of Insect Pests

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The use of cheap pesticides during the 20th century reduced the demand for resistance in crops, with the result that intrinsic anti-herbivore defenses have been lost from many crops. However, with growing public awareness of the harmful side effects of pesticides, and in turn their increasing regulation, crop production is gradually shifting from heavy pesticide-dependency towards integrated pest management (IPM). Two central goals of IPM are to restore strong intrinsic resistance in crop plants, and to facilitate biological control using natural enemies (Rodriguez-Saona *et al.*, 2011; Chen *et al.*, 2015; Stenberg *et al.*, 2015). However, the dependance of biological control agents on the quality of pest prey or host implies that such interactions could also depend on plant quality. Thus, altering plant quality traits like resistance can affect the performance and behavior of predators and parasitoids (Wilder and Eubanks, 2010; Bukovinszky *et al.*, 2012; Stenberg *et al.*, 2012), and therefore lead to potential conflicts between IPM goals.

In this study a collection of wild germplasm of woodland strawberry, *Fragaria vesca* L. (Rosaceae) was used as model system to examine the occurrence of intrinsic plant resistance against herbivores as well as to investigate plant-mediated effects on performance of natural enemies (Fig. P19.1). In Scandinavia (Olofsson and Pettersson, 1992; Stenberg and Axelsson, 2008; Stenberg, 2012), the Baltic states (Kaufmane and Libek, 2000), and Russia (Bulukhto and Tsipirig, 2004) organic strawberry plantations can suffer from severe outbreaks of the strawberry leaf beetle, *Galerucella tenella* (L.) (Coleoptera: Chrysomelidae), leading to feeding damage on leaves and flowers (Muola *et al.*, 2017). The main natural enemy of the herbivore is the parasitoid *Asecodes parviclava* (Thomson) (Hymenoptera: Eulophidae) (Stenberg, 2012, 2014).

Based on preliminary data indicating that wild *Fragaria* show high intraspecific genetic variation in intrinsic herbivore defenses (Cole and Ashman, 2005; Stenberg unpubl. Data), we screened over eighty wild woodland strawberry genotypes for resistance (antibiosis) against *G. tenella*. Measuring herbivore developmental time and pupal weight, we compared *Galerucella* larvae that had been reared individually on detached leaf material from different *Fragaria* genotypes. Plant genotype had significant effects on both herbivore development time and pupal weight, thus indicating intraspecific variation in plant resistance among the screened *Fragaria* genotypes.

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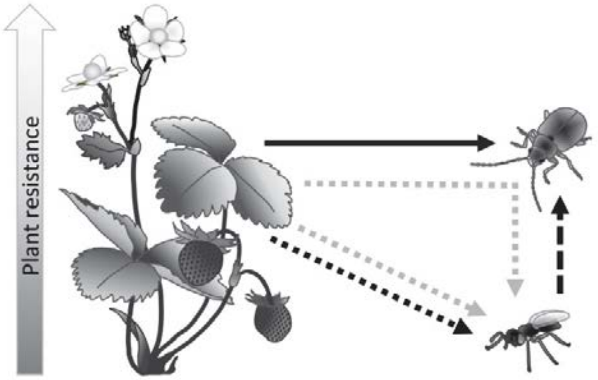

Fig. P19.1. A conceptual diagram of how plant resistance affects interactions between the strawberry, strawberry leaf beetle, *Galerucella tenella*, and its parasitoid, *Asecodes parviclava*. Herbivory by the leaf beetle can either be decreased directly by plant resistance (black arrow) and/ or by releasing natural enemies (black long dashed arrow). Plants can assist biological control by attracting natural enemies when attacked (black dashed arrow). Plant resistance can indirectly influence biological control by either altering the host quality or attraction of the parasitoid to the host (gray dashed arrows).

Using larval developmental time and pupal weight as proxies for plant resistance (as obtained in the above experiment), we selected a subset of resistant and susceptible genotypes for further studies of effects of resistance on the parasitoid *A. parviclava*. The selected plant genotypes were used to test whether variation in plant resistance can modulate the life history traits of the parasitoid. In order to test this, first stage larvae of *G. tenella* were parasitized individually, and fed with detached leaves from the selected genotypes until pupation. The results showed that both mummification rate and developmental time (from parasitization to pupation) were significantly affected by plant genotype, but that strong plant resistance did not have an effect on the parasitoid. Although several resistant genotypes facilitated a high mummification rate – meaning that the parasitized larvae turned successfully into a parasitoid pupae – the division of genotypes into susceptible and resistant groups based on the beetle performance had no effect on parasitoid performance.

As a whole, our study revealed that both the *G. tenella* and its parasitoid were affected by plant genotype, showing that the genotypes differ in their quality as food source. The beetle and the parasitoid were differentially affected, however, indicating that the parasitoid is affected by different plant quality traits than the beetle. Beetle performance alone therefore seems to be an insufficient proxy for parasitoid performance, and further studies are needed to elucidate the plant trait(s) affecting the parasitoid. Optimizing plant traits to support tritrophic interactions is still uncommon in plant breeding programs, thus the insights gained through this project may help to open up opportunities for breeding strawberry varieties that facilitate improved biocontrol of herbivores, and help to reintroduce ecologically important genes back into commercial varieties.

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Poster 20: Promoting *Cotesia rubecula*, an antagonist of *Pieris rapae*

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The loss of natural habitat through intensification of agriculture, negatively impacts the existence of many arthropod predators and parasitoids (Naranjo and Ellsworth, 2009; Bommarco *et al.*, 2011; Winqvist *et al.*, 2012). To counteract this trend, the general biodiversity of crop fields can be increased by extensively managing their bordering areas, e.g., by sowing tailored flowering strips, which provide food and shelter for many insects (Moonen and Bàrberi, 2008; Plecas *et al.*, 2014; Gaigher *et al.*, 2015; Inclan *et al.*, 2015a, 2015b). Desired conservation biological control effects can be reached, when flowers are carefully selected to promote the beneficial insect of interest, but not the pest (Tooker and Hanks, 2000; Vattala *et al.*, 2006). *Pieris rapae* (L.) (Lepidoptera: Pieridae) is a pest of the brassica family, mostly attacked by the solitary larval parasitoid *Cotesia rubecula* (Marshall) but also by the gregarious larval parasitoid *Cotesia glomerata* (L.) (Hymenoptera: Braconidae). The enhancement of *C. rubecula* may reduce *P. rapae* population densities, resulting in less crop damage and higher economic gains. Therefore, laboratory and field trials were conducted to test the suitability of selected flowers to attract, enhance and retain the beneficial insect *C. rubecula* in brassica fields.

Laboratory trials were conducted to assess the influence of *Centaurea cyanus* L. (Asteraceae), *Fagopyrum esculentum* Moench (Polygonaceae) and *Vicia sativa* L. (Fabaceae) on the longevity of *C. rubecula*, whereby a negative control was included in which wasps were only provided with water. This was tested by adding newly hatched (< 24 h) wasps to the treatments and recording their survival on a daily basis. Compared to the negative control, wasps survived longest with *F. esculentum* followed by *C. cyanus* and *V. sativa*.

Further, olfactometer experiments were performed to determine the attractiveness of the selected flowers towards the parasitoid *C. rubecula*. This was done using a Y-tube olfactometer as described by Belz *et al.* (2013). Unfed virgin female wasps (< 24 h) were given the choice between a flower source and air or between two different flower sources. *C. cyanus* turned out to be the most attractive flower to *C. rubecula.*

Field trials were conducted to estimate the potential of these flowers in enhancing the parasitation rate of *P. rapae* larvae in brassica fields. We tested the following three treatments: flowering strip and companion plants (*C. cyanus*), flowering strip, and a control without addition of flowers (Fig. P20.1). Whole fields were used as true replicates. The parasitation rate was monitored by weekly exposing and recollecting *P. rapae* larvae in exposure sets placed between brassica plants in the insecticide free data collection area

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(Fig. P20.1). Each set consisted of a pot filled with soil and a cabbage plant. The overall recollection rate was around 50 %. To assess whether the recollected *P. rapae* larvae were parasitized by their main larval parasitoid *C. rubecula* or rather by *C. glomerata*, a triplex qPCR reaction was developed with specific primers and fluorogenic TaqMan probes for the detection of the host and both parasitoids simultaneously in the same reaction. Higher parasitism rates were found in fields provided with companion plants and flowering strips compared to fields without flower sources or only flowering strips.

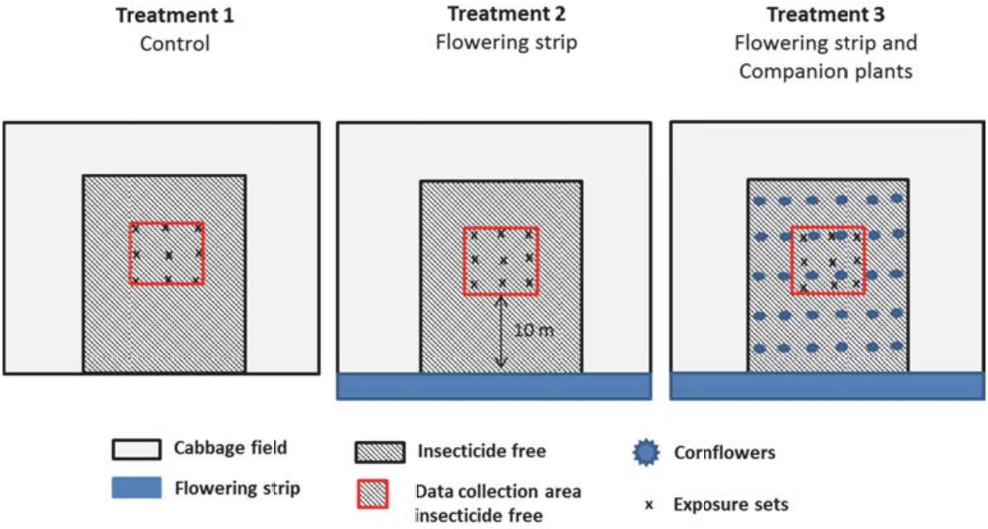

Fig. P20.1. Experimental design of the field trials. Three different treatments were tested in cabbage fields to assess the influence of flowers on parasitism rates of *Pieris rapae* larvae. In each field an insecticide free area was defined (dark grey). Data were collected in a plot within this area. **Left**: Field without flowers. **Middle**: Field with flowering strip. **Right**: Field with a flowering strip and rows of alternating cabbage and cornflowers within the insecticide free plot.

With the present and ongoing work we aim to demonstrate, if and how well selected flowers can be used to attract and enhance the parasitoid *C. rubecula,* not only under laboratory conditions, but also in the field.

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Poster 21: Cold Acclimation Increases Cold- and Starvation Tolerance but Reduces Predation Rate and Reproduction in the Predatory Mite *Gaeolaelaps aculeifer*

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Predatory arthropods used in biological control are produced under optimal thermal conditions in the laboratory before release in the field. In northern Europe, however, temperatures in the field are often low compared to rearing temperatures of the biocontrol agent, and the predators are therefore not thermally acclimated to field conditions if released directly after rearing at their optimal growth temperature. Ectotherms respond to their thermal environments by physiological acclimation which increases tolerance to thermal extremes, and a period of thermal acclimation to colder temperatures might therefore increase performance and control efficiency under low field temperatures. However, acclimation to suboptimal temperatures may increase some performance parameters that increase overall fitness at the expense of others, and different performance parameters may therefore trade-off under different thermal conditions. We investigated effects of cold acclimation on several performance parameters in the predatory mite *Gaeolaelaps aculeifer* (Canestrini) (Mesostigmata: Laelapidae), which is used in biological control against a range of soil-dwelling pests.

We acclimated adult female *G. aculeifer* to low (10°C), intermediate (15°C), or standard rearing (20°C) temperatures by placing them in different climate controlled rooms for seven days. We then tested subsets of mites from all three acclimation temperatures at each of the same three test temperatures for predation rate, reproduction, starvation tolerance, and locomotor activity. Another set of mites were acclimated at each of the three temperatures over four days including an additional group at 5°C, and survival was assayed following a 24h exposure to -2°C.

Our results showed higher cold tolerance in mites acclimated to lower temperatures, with 80% survival in mites acclimated to 10°C, 50% survival in mites acclimated to 15°C, and 20% survival in mites acclimated to 20°C. Only, at the lowest acclimation temperature (5°C), was survival equal to that of mites acclimated to 20°C, indicating that 5°C is too low of an acclimation temperature.

Mites survived starvation longer at low temperatures, but also tolerated starvation better after acclimation to low temperature across all test temperatures. In contrast, prey consumption, eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), and mite egg production were higher at higher test temperatures, and also higher across all test temperatures in mites that were acclimated to high temperature. Whereas stress tolerances were upregulated after low temperature acclimation, consumption and reproduction were

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thus downregulated after low temperature acclimation. Locomotor activity was higher at higher test temperatures, but not affected by acclimation temperature. The results show that acclimation to cold incurs a change in the physiological trade-off balance so some performance parameters are enhanced while others are diminished.

Poster 22: Mass-rearing Optimization of the Parasitoid *Psyttalia lounsburyi* for Biological Control of the Olive Fruit Fly

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The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), discovered in 1998 in California, is a direct pest of olives that has invaded the Mediterranean Region and California (Rice *et al.*, 2003; Zalom *et al.*, 2009). The fly is believed to have originated from Africa (Hoelmer *et al.*, 2011), and *Psyttalia lounsburyi* Silvestri (Hymenoptera: Braconidae), a larval parasitoid from Africa, has been approved for release in the USA as a classical biological agent (Copeland *et al.*, 2004; Daane *et al.*, 2015). *P. lounsburyi* oviposits on *B. oleae* larvae inside the olive fruit, and completes development after the host pupates. However, it has been difficult to rear the parasitoid in the laboratory because it is multivoltine, and the host develops only in fresh olives, which are not available for most of the year (Daane *et al.*, 2008). A method to rear the parasitoid on the factitious host, Mediterranean fruit fly, *Ceratitis capitata* (Wiedemannn) (Diptera: Teprhitidae), which can be reared on artificial diet throughout the year, was developed by Thaon *et al.* (2009). Although functional, this method needed to be improved to allow mass production indispensable for release in the field. Thus, we developed in our laboratory a number of ways to improve the efficiency of rearing by focussing on increasing productivity per female and reducing human labour.

Thaon *et al.* (2009) developed an artificial 'olive' to stimulate the oviposition behaviour of *P. lounsburyi* female (Figs. P22.1 and P22.2). To create an “exposure ball”, a 2 cm-diameter polystyrene ball is completely covered with 2-mm thick layer of larval artificial diet. Seven-day-old larvae (third instar) of *C. capitata* are then placed all over the ball and maintain with a stretched sheet of Parafilm™. After a period of exposure, the “ball” device is removed from the parasitoid cage and larvae are placed in a larger aerated box with artificial diet until pupation. The parasitoids start emerging 21 and 23 days after parasitisation for males and females, respectively. To improve the use of this method and optimise production, we first determined the optimum duration of the period of exposure (3 hours instead of 24). We also estimated the best reproduction period for female parasitoid, which is between the first and the fifth week post-emergence. Thus, hosts are exposed 10 to

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15 times to 1-2-week old mated females over a three-week period (instead of nine, i.e., during their whole life).

Fig. P22.1. Adult *Psyttalia lounsburyi* rearing cage. (photo: B. Hurtrel).

Fig. P22.2. A 5-mm diameter spherical shaped device for exposure of C*eratitis capitata* larvae to *Psyttalia lounsburyi.* (photo: A. Blanchet).

The effect of high density (overcrowding) on reducing fecundity of entomophagous insects has long been known (Campos and Gonzales, 1991; Watt, 1960). For mass rearing, it is essential to be able to produce the maximum of descendants with the lowest number of breeder individuals. The previously applied method in our laboratory consisted of using a cohort of 200 parasitoid females and 100 males in a cylindric cage (25 cm diam., 20 cm length; i.e., 32.7 cm^3 per wasp; Fig. P22.1). In order to reduce intra-specific competition, we reduced this density by putting 50 females and 25 males per cage (i.e. 130.8 cm^3 per wasp). Thanks to this change, the number of descendants per female for one exposure increased by 48% (a mean of 2.11 descendants per female for 50-female cohorts vs. 1.42 for 200-female cohorts).

In biological control programs using parasitoids, females are more valuable than males because males can fertilize multiple females, and only females directly kill the target pest. By reducing density, we were also able to improve the female sex ratio by 45% (increasing females from 33.1% to 47.9%).

In order to standardize the *P. lounsburyi* rearing conditions, it was first essential to determine the number of fly larvae to expose to provide an *ad libitum* resource to parasitoids. Secondly, we had to find a simple and rapid method to collect and expose the chosen number of *C. capitata* larvae. For this study, we used samples of 7-day-old *C. capitata* larvae collected from “exposure balls” exposed to parasitoids. We measured the volume and the weight of each sample, and counted the number of individuals. We concluded that 1 ml of larvae contains about 662.4 individuals. Thus, for a cage containing 50 *P. lounsburyi* females with one exposure ball covered with 2 ml of larvae, there are about 26 host available per female. The maximum number of descendants per female during a 3 hours oviposition period was 5. So, 2 ml of larvae easily provided an "ad libitum" condition for parasitoid oviposition.

To standardize the use of a chosen amount of fly larvae for exposure, we created a milliliter "dip net" made of a Falcon milliliter tube (15 ml tube with conic bottom). The bottom of the tube was cut and a thin nylon mesh was fixed with hot glue on one end, which permits scooping larvae from water (the simplest method of handling) while allowing the liquid to pass through. A plastic handle was also fixed to the device for easier handling (Fig. P22.3).

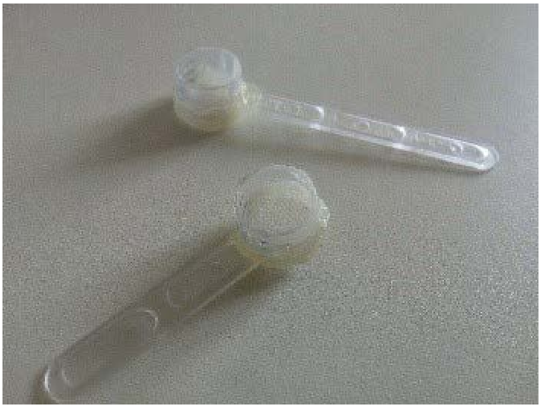

Fig. P22.3. Milliliter "dip net" to measure 2ml of fly larvae. (photo: F. Chardonnet).

Counting and sexing *P. lounsburyi* adults in preparation for shipment was one of the most time-consuming tasks during mass production (limiting the increase of production). In order to reduce this workload, we tested the reliability of using subsamples of about 10% of the fly pupae produced (after exposure to parasitism), from which the total number of emerged parasitoid adults is estimated. We counted the total number of emerged individuals and compared this number with the estimation made from the 10% sample. Because the estimate did not significantly differ from the real number of parasitoids collected, we considered that using a subsample of 10% of pupae was an accurate method to estimate adult production. This reduced counting time by 90 %, saving time which could be used to further increase production.

Thanks to all these modifications, we significantly improved the number of progeny produce per female, and the female sex ratio of progeny. We also improved conditions for rearing larvae and holding emerged adults until release and thus improved survivorship of both immatures and adults. In 2016, we were able to produce over 54,000 adults, and shipped over 22,900 for release in California.

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Poster 23: Evaluation of Sixspotted Thrips, *Scolothrips sexmaculatus*, for Biological Control of Spider Mites in California Almonds

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Sixspotted thrips, *Scolothrips sexmaculatus* (Pergande) is one of the oldest species of thrips known from North America (Bailey, 1939). It is recognized as a specialist predator of spider mites whose field biology has been poorly understood for nearly a century. Published laboratory studies on sixspotted thrips in the 1970s suggest that adults can live 29 to 67 days depending on temperature with a generation time of 18 to 51 days (Gilstrap and Oatman, 1976; Coville and Allen, 1977). Laboratory studies also document that female thrips can eat 7 to 50 spider mite eggs per day, can eat 38 immatures or 16 adults per day, and can lay 90 to 166 eggs over their lifespan. When these laboratory data are combined, calculations show that sixspotted thrips can have population doubling times ranging from 2.7 to 8.7 days at temperatures that are typical in California almond orchards during the summer when spider mites are prevalent.

These laboratory data suggest that sixspotted thrips has great potential to provide biological control of spider mites under field conditions in California almonds. During the 2016 and 2017 growing seasons we conducted field research to document periods of the year when sixspotted thrips are present, population growth rates under field conditions, and relationships between pest and prey populations under unsprayed field conditions. Trials also evaluated 7 different colors and sizes of sticky cards that have potential for use by pest control advisors making field decisions about management programs.

The yellow strip trap (Great Lakes IPM) was the most effective trap for monitoring sixspotted thrips, capturing >5X more thrips that the other six cards tested. Weekly captures for the yellow strip trap exceeded 700 thrips per week compared to less than 150 thrips per week for the other six cards. The yellow strip trap was also superior to the other six traps when data were converted to thrips per square area to account for differences in the sizes of the cards.

Use of the yellow strip trap in subsequent studies showed that there is a significant relationship between spider mites and sixspotted thrips in commercial orchards, and that sixspotted thrips can provide complete biological control when conserved (Fig. P23.1). For example, in one field study spider mites increased from 4.8 to 16.2 per leaf from 14 Jul to 11 Aug with a population doubling time of 15.9 days. However, over the same period of time sixspotted thrips increased from 0 to 970 per sticky card per week with population doubling times of 4.2 days. By 25 Aug, mite populations had crashed to near zero while sixspotted thrips per card per week increased to over 2,700. The yellow sticky card also

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caught adult spider mite destroyer beetles, *Stethorus picipes* Casey (Coleoptera: Coccinellidae). Similar relationships between predators and prey were also seen at other research sites, suggesting significant potential for conservation biocontrol as a viable method for spider mite management.

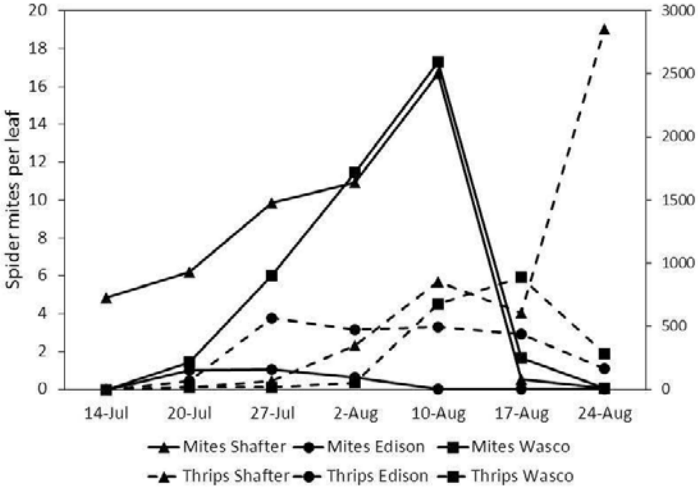

Fig. P23.1. Yellow strip trap counts of spider mites and sixspotted thrips in commercial almond orchards in California.

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Poster 24: Area Wide Integrated Pest Management Incorporating the Sterile Insect Technique: Gut Microbiota Impacts on Tephritid Fitness and Performance

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The Queensland fruit fly (Q-fly), *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), is native to Australia, and is a pest and biosecurity threat to its AU$9 billion horticultural industries (HIA, 2015). Q-fly is controlled using a range of tools including the Sterile Insect Technique (SIT), a sustainable, target-specific biological form of pest management. The success of SIT relies upon mass-reared sterile males mating with wild females upon release. Mass-rearing, domestication and irradiation of pupae in SIT programs have been found to reduce the fitness and quality of the released sterile insects (Collins and Taylor, 2011). The midguts of individual wild and domesticated larvae were analysed with Next Generation Sequencing (NGS). Wild larvae were sourced from two locations in New South Wales, Australia and domesticated larvae from three different facilities within New South Wales.

Following *B. tryoni* larval midgut molecular characterisation, we selected four bacterial candidates isolated from the midguts of wild larvae for their potential as 'probiotics' to improve the fitness and performance of mass-reared larvae and adult flies. Single strains of live bacteria from the families Acetobacteraceae, Enterobacteriacae, Lactobacilliaceae, and Leuconostocaceae were added added individually to the *B. tryoni* larval carrot diet (Fig. P24.1). A treatment consisting of a blend of all four of the candidates together was also included.

Relative abundance and diversity of gut bacteria were higher in wild larvae compared to domesticated larvae. Sequences identified as *Asaia* sp. (Acetobacteraceae) dominated in both wild and domesticated larvae. Wild larvae were also dominated by Leuconostocaceae, Enterobacteriacae and other genera within the family Acetobacteraceae.

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Fig. P24.1. Second instar larva of *Bactrocera tryoni* feeding on bacteria enriched carrot diet. Scale = 5 mm.

Developmental and fitness parameters were measured including larval development time, and adult survival in field cages. The bacterial treatments had varying effects, depending on the bacterial treatment added. Candidates from Enterobacteriaceae and Acetobacteriaceae reduced larval development time, however they also reduced the field longevity of adult flies, compared to the no bacteria added control. The candidates from Lactobacillaceae and Leuconostocaceae, both from the order Lactobacilliales, delayed larval development and increased field longevity of adult flies. The blend slightly delayed larval development time and field longevity compared to the control.

The NGS findings suggest the gut bacterial communities of Q-fly larvae are affected by the process of domestication and mass-rearing, with higher diversity present in wild larvae. Bacterial probiotics fed to mass-reared larvae, have the potential to positively influence Q-fly larval and adult quality characteristics. Shortened development times and increased longevity of probiotic fed flies can reduce mass-rearing costs and improve the efficiency of AWIPM programs that incorporate SIT. However, selecting the right bacterial candidates to feed larvae is key, as some have positive effects and others negative depending on the fly trait. While these studies have implications for fertile *B. tryoni* (e.g., colonies in mass-rearing facilities), future studies need to test the effects of probiotic fed larvae on post-irradiated sterile males.

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Poster 25: Non-target Impacts of *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) on Natural Enemies of Arthropod Pests

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A number of biopesticides based on entomopathogenic fungi have been developed since the 1960s. The most common mycopesticides are based on *Beauveria bassiana* (Balsmo-Crivelli) Vuillemin, *Metarhizium anisopliae* (Metchnikoff) Sorokin, *Isaria fumosorosea* (Wize), and *B. brongniartii* (Saccardo) (Clavicipitaceae) (Faria and Wraight, 2007). While the efficacy of these products against target pests has been documented in many studies, less is known about their effects on beneficial organisms. The objective of the present study was to assess non-target impacts of *I. fumosorosea*, a globally distributed species naturally occurring in soil samples and reported from many countries. It has a relatively wide host range across several insect orders. Various strains have been successfully used in biological control and several commercially produced mycopesticides based on them have been developed in America, Asia or Europe (Zimmermann, 2008).

Material and methods: Laboratory bioassays were conducted to reveal the effect of *I. fumosorosea* on hymenopteran parasitoids of the horse chestnut leaf miner, *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae), predatory mites *Typhlodromus pyri* Scheuten and *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), and the entomopathogenic nematode *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae). We used strain CCM 8367 originating from the Czech Republic (Prenerová *et al.*, 2013) which was found to be highly virulent against several pest species (Hussein *et al.*, 2013; Hussein *et al.*, 2016; Zemek *et al.*, 2016). Conidiospores and blastospores of the fungus were obtained by cultivation on Sabouraud agar and by submerged cultivation using an orbital shaker, respectively. Suspension of spores at a concentration of 5×10^7/ml with 0.02% soaking agent Tween® 80 was used in the trials.

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Results and discussion: The results revealed that on average 22.5 hymenopteran parasitoids emerged from 200 g of fallen horse chestnut leaves treated with *I. fumosorosea* CCM 8367 while 99.6 parasitoids emmerged from control leaves under laboratory conditions. The difference was highly significant (t=6.7144, df=18, $P<0.0001$). Under field conditions, the fungus reduced populations of overwintering parasitoids by 46%. The same fugus species was reported to infect the aphid parasitoid, Aphelinus asychis Walker (Hymenoptera: Aphelinidae) (Lacey *et al.*, 1997).

The fungus turned out to be pathogenic also to phytoseiid predatory mites. Conidiospores in contact with cuticle of *T. pyri* females germinated (Fig. P25.1) and caused mortalities of 14.3 and 37.1% at 72 and 144 hours after the treatment, respectively, while in the control, treated with distilled water mixed with Tween® 80, the mortalities were 1.4 and 5.7%, respectively. The effect of fungus treatment was significant (Fisher's test, P=0.0088 and $P<0.0001$). *Isaria fumosorosea* was also pathogenic to *P. persimilis* and development of mycosis was seen on its cadavers (Fig. P25.1). Both predatory mite species were, however, less susceptible to the fungus than spider mites (Zemek *et al.*, 2016). Seiedy *et al.* (2015) showed that *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) was susceptible to *B. bassiana* when conidia were applied directly to the mites, causing mortality upto almost 50%. In contrast, Wu *et al.* (2014) never observed *B. bassiana* penetration of *Neoseiulus* (*Amblyseius*) *barkeri* Hughes (Acari: Phytoseiidae) cuticle and no pathogenicity of this fungus to the predatory mite was found.

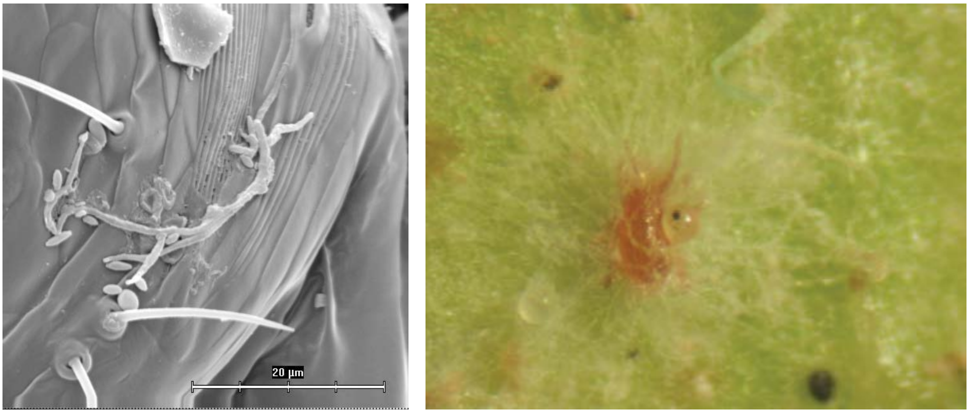

Fig. P25.1. Scanning electron micrograph of *Typhlodromus pyri* cuticle with attached conidia of *Isaria fumosorosea* forming germ tubes (**left**) and mycosis developed on a *Phytoseiulus persimilis* cadaver (**right**).

Our results further demonstrate that *I. fumosorosea* has a negative impact on *S. feltiae*. Although it is not pathogenic to the nematode, when the host (Colorado potato beetle) was colonized by fungus, only a part of the nematodes developed to adults, and in some cadavers, only dead *S. feltiae* adults were observed. Furthermore, adult body size of nematodes developing inside the cadavers was lower in comparison to the control. The body length of *S. feltiae* females was also negatively correlated with duration of fungus infection ($R^2=0.0941$; N=80; P=0.0056). This could be due to the anti-bacterial activity of some *I. fumosorosea* metabolites which might affect the nematode's symbiotic bacteria, *Xenorhabdus bovienii* Akhurst and Boemare (Enterobacteriaceae). Such negative interactions were observed, for example, between *B. bassiana* and *Steinernema ichnusae* Tarasco, Mráček, Nguyen & Triggiani (Rhabditida: Steinernematidae) (Tarasco *et al*., 2011).

We can conclude that *I. fumosorosea* CCM 8367 might have negative impact at least on some species of beneficial organisms and that such side-effects should be taken into account when this entomopathogenic fungus is implemented in biological control.

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Poster 26: Releases of *Trissolcus japonicus* and *Anastatus* sp. for Suppression of *Halyomorpha halys* in Kiwifruit Orchards

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Kiwifruit is one of the most important cash crop in Shaanxi Province, China, and recently has been plagued by occasional outbreak of *Halyomorpha halys* Stål (Hemiptera: Pentatomidae). In order to avoid using broad-spectrum insecticides, biological control approaches to control *H. halys* are needed. *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) and *Anastatus* sp. (Hymenoptera: Eupelmidae) are two dominant egg parasitoids of *H. halys* in northern China. Inoculative releases of T. *japonicus* and augmentative releases of *Anastatus* sp. were made twice in 2016 in a kiwifruit orchard at Zhouzhi County. Parasitism by *T. japonicus* on sentinel eggs of *H. halys* reached 79% and 77% in *T. japonicus* and *Anastatus* sp. release plots after second release, while parasitism by *T. japonicus* was 76% in no-release plots. The higher parasitism rates in both *Anastatus* sp. release and no-release plots might be attributed to dispersal of *T. japonicus* from the first release. Moreover, no parasitism by *Anastatus* sp. on sentinel eggs of *H. halys* was observed in both release and no-release plots, which might be due to short exposure period of host sentinel eggs that *Anastatus* sp. were largely at pre-oviposition stage. Field trials will be repeated in 2017 to further assess the effectiveness of releases of the two parasitoids for suppression of *H. halys* in kiwifruit orchards.

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Poster 27: Caught on Camera: Confirmation of Natural Enemies Attacking Pest Leafrollers in Kiwifruit Orchards

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We identified parasitoids and predators of pest leafroller species, *Ctenopseustis obliquana* (Walker) and *Cnephasia jactatana* Walker (Lepidoptera: Tortricidae), in the shelterbelts of 12 kiwifruit orchards over two years. Parasitoid identity and parasitism rates were determined using larvae placed on the leaves of potted poplar trees, and predators were identified by videoing tethered larvae and egg batches. Predation was measured using the disappearance rate of sentinel larvae and egg batches. We identified 14 predators and four parasitoid species, however, initial results suggest parasitism and predation rates were low - a maximum of 3% and 35%, respectively. Increasing the contribution of these species to the control of these pests may reduce the number of Bt-based insecticides that need to be applied by growers.

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Poster 28: *Telenomus* sp., a Potential Biocontrol Agent Against the Cabbage Moth *Mamestra brassicae*

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The cabbage moth, *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae), is an insect pest distributed in Europe and Asia. Its larvae (Fig. P28.1, left) can cause severe damages to different brassica crops and alternatives to conventional insecticides are scarce. To design a new biocontrol agent the right natural enemy should be chosen based on the crop of interest (Parra 2010a). Field trials in Switzerland showed that parasitization rates through released and naturally occurring *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) were lower than those of naturally occurring *Telenomus* sp. (Hymenoptera: Scelionidae) (Pfiffner *et al.*, 2009; Balmer *et al.*, 2013) (Fig. P28.1, right).

Fig. P28.1. Larva of the cabbage moth *Mamestra brassicae* feeding on cabbage leaves (**left**, photo: H. Luka) and a *Telenomus* sp. female parasitizing *M. brassicae* eggs (**right**, photo: C. Daniel).

Parasitism rate and proportion of females produced are two important aspects determining the success of a parasitoid rearing (Parra, 2010b). As shown for other *Telenomus* spp., these aspects can be influenced by several factors, like temperature (Legault *et al.*, 2012; Pomari *et al.*, 2012), competition between parasitizing females

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(Carneiro *et al.*, 2009) and the time between emergence and the first parasitization attempt (Charnov and Skinner, 1985). In this study, laboratory trials were conducted to understand the effects of these rearing conditions on *Telenomus* sp., in order to develop a small-scale rearing of this parasitoid.

Under field conditions, the number of females parasitizing the same egg clutch is limited to a few individuals (Carneiro *et al.*, 2009). In mass-scale rearing, intraspecific competition could possibly affect the success of the rearing unit. In our study, we tested two female densities: three females per rearing unit (standardized number of eggs) and one female per rearing unit. Based on the Local Mate Competition (LMC) theory of Hamilton (1967) we predict a negative influence of the higher female density on the proportion of female produced. On the other hand, we can expect to reach a higher parasitization rate when more than one female are allowed to parasitize the same egg clutch.

We tested temperatures ranging from 20 °C to 30 °C, as this range represents the temperatures measured in Swiss cabbage fields during summer 2015. The developmental time of other *Telenomus* species was proven to depend on temperature (Bueno *et al.*, 2008; Legault *et al.*, 2012; Pomari *et al.*, 2012), the same is expected to be true for *Telenomus* sp. Temperature could also influence the successful emergence of the progeny, indicating that there could be processes impairing the development of the parasitoid egg inside the host egg. However, further research is needed to better understand the interaction between *Telenomus* sp. and *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae) under different temperature regimes.

We investigated the life expectancy of *Telenomus* sp. in the presence or absence of host eggs and, as the parasitization process is energy demanding for females, we expect females to live longer in the absence of eggs than in their presence. The influence of different egg deprivation periods on the longevity and fecundity of the parasitic wasp was examined. *Telenomus* sp., just like other egg parasitoids (Boivin, 2010), is moderately synovigenic. We therefore expect egg deprived females, which did not have contact with host eggs for a certain time before parasitization, to show a higher parasitization performance than females that were not egg deprived.

Our results could help building a stable rearing of *Telenomus* sp., potentially allowing the development of a mass rearing.

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Poster 29: Alteration of Predatory Behavior of a Generalist Predator by Exposure to Two Insecticides

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Spiders significantly reduce pests' populations in many agroecosystems (Marc *et al.,* 1999). Predation pressure of spiders on pest depends on their prey choice as well as predatory activity (Michalko and Pekár, 2017). However, both traits may be altered by an exposure to the pesticides that can decrease or increase predatory activity of spiders (Pekár, 2012). However, the effects of pesticides on a prey choice have not been studied in the natural enemies yet. The prey choice in generalist spiders might be altered e.g., by the blurred cognitive abilities and/or increased appetite (Pekár, 2012).

We studied how the application of pesticides can alter the prey choice and predatory activity of the spider *Philodromus cespitum* (Araneae: Philodromidae) that belongs to the most abundant spiders in orchards in Central Europe (Bogya *et al.*, 1999). We tested the effect of two insecticides, SpinTor® (a.i. spinosad) and Integro® (a.i. methoxyfenozide). We investigated the prey choice between the pest *Cacopsylla pyri* (Hemiptera, Psyllidae) and spiders *Theridion* sp. (Araneae: Theridiidae).

We divided the philodromids to the three treatments randomly: water as a control, insecticide SpinTor® (concentration = 0.06%), and insecticide Integro® (concentration = 0.08%). After the exposure, the spiders were placed in Petri dishes individually. Three *Cacopsylla* and three *Theridion* were then introduced in each dish simultaneously. The number of killed prey was checked every 15 min for three hours.

The prey choice of philodromids was altered by exposure to the both pesticides, Integro® and SpinTor®. Philodromids preferred *Theridion* to *Cacopsylla* in control treatment. In contrast, philodromids did not possess any distinct prey preferences in the pesticide treatments. The application of both pesticides also increased the short term capture rate. However, this was caused by increased predation on *Cacopsylla* while the predation rate on *Theridion* did not differ among the treatments.

The changes in prey choice and capture rate might be caused by the altered cognitive abilities, increased voracity, or modified appetite of the philodromids. The incapability to evaluate the prey characteristics properly may lead to the severe fitness consequences due,

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e.g., nutritional imbalance or intoxication (Toft, 2013). The results also indicate that the exposure to the two insecticides can alter food-web dynamics by modifying the predatory traits of spiders (Michalko and Pekár, 2017).

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Poster 30: Impact of Plant Extracts of *Embelia ribes* and two Commercial Pesticides on Mortality and Predator Activity of a Generalist Predator, *Oxyopes lineatipes*

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Spiders are among the most abundant predators in various agroecosystems, and they comprise a substantial portion of pests' natural enemies (e.g., Marc *et al.*, 1999). Pesticides can influence biocontrol agents in various ways, notably by affecting their functional responses and by reducing their predatory function (Pekár, 2012; Michalko and Košulič, 2016). To maximize the beneficial effect of spiders in agro-ecosystems and to reduce environmental pollution, it is important to use pesticides that have high efficacy on target pests, but minimal side effects on non-target organisms. Biopesticides appear to be promising new substances for controlling pests due to their natural properties and high effectiveness in inhibiting pests (Biondi *et al.*, 2012). Nevertheless, their side effects on nontarget organisms must be evaluated before they can be included in Integrated Pest Management systems. Here, using *Oxyopes lineatipes* (Koch) (Aranae: Oxyopidae), a common generalist predator that has a role as a potential biocontrol agent of various pests in agroecosystems across Southeast Asia (Barrion *et al.*, 2012), we aimed to evaluate lethal and sublethal effects of newly introduced biopesticides from plant extracts of *Embelia ribes* Burman (Ericales: Primulaceae), a commercial natural product (azadirachtin) and a synthetic acaricide (amitraz).

Specimens of *O. lineatipes* were collected from *Jatropha curcas* (Euphorbiales: Euphorbiaceae) plantations in experimental farm, Nakhon Pathom, Thailand, by sweeping from herb vegetation at the end of June 2014. We collected spiders of different sizes inasmuch as the *O. lineatipes* populations are composed of spiders of various sizes throughout the entire year in tropical ecosystems and we were interested in the possible size-specific effects of the pesticides on adult and subadult spiders.

To compare the agrochemicals according to the mortalities they cause in spiders, we tested the concentrations recommended for field treatments (i.e., 0.004% for amitraz; 1.25 x

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10−4%; 5 x 10−4% for azadirachtin; 0.5% for plant extracts from *Embelia)*. We used two control groups treated with the solvents used for the pesticides' dilutions: water and 10% ethanol. Further, in order to find the lethal concentrations (LC_{50s}), the pesticides were assayed at five concentrations (*Embelia*: 0.5%, 0.75%, 1%, 1.5%, and 2%; amitraz: 0.002%, 0.004%, 0.01%, 0.02%, and 0.04%; azadirachtin: 25 x 10^{-4}%, 5 x 10^{-4}%, 1 x 10^{-4}%, 1.25 x 10^{-4}%, and 5 x 10^{-4}%).

For the sublethal bioassay, we tested only the lower recommended field doses for azadirachtin (1.25 x 10^{-4}%) and *Embelia* inasmuch as amitraz had caused almost 100% mortality in the studied subadult and adult spiders. For *Embelia*, we used almost double the recommended dose (0.75%) because the LC_{50} for predacious beneficial mites is 0.67%, while for broad mites it is 0.83% (Vichitbandha and Chandrapataya, 2011).

We found that the spider's mortality increased with rising concentration of commercial products, azadirachtin and amitraz, but not with rising concentration of the extracts from *E. ribes*. Amitraz caused the highest mortality, almost 100% spider mortality at recommended doses. This treatment also caused higher mortality with increasing size of spiders (not in azadirachtin and *Embelia*).

Concerning sublethal effect of studied pesticides, azadirachtin significantly lowered the functional response of *O. lineatipes* while there was no significant difference in functional responses of spiders from the control and *E. ribes* treatments. The studied biopesticides had much lower or no significant effect on subadult and adult specimens of *O. lineatipes* in comparison to amitraz. *Embelia* extracts appeared to compatible with sustainable plant protection programs, due to its high efficiency in pest suppression (e.g., Noosidum and Chandrapatya, 2015), but no adverse effects on natural enemies. In contrast, Azadirachtin that is considered as safe for non-target organisms (Stark, 2013) exerted significantly negative sublethal and slight lethal effect. We therefore point out that ecotoxicological studies should not focus solely on mortality testing on non-target natural enemies, even if the candidate pesticides are a priori harmless to natural enemies.

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Poster 31: *Vespula* Biocontrol in New Zealand Revisited

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Social wasps are a pest in many temperate regions of the world, and much research effort has been invested in developing control strategies (Beggs *et al.*, 2011). In New Zealand, where there are no indigenous social wasps, introduced *Vespula* species have become invasive.

The German wasp, *Vespula germanica* (Fabricius) (Hymenoptera: Vespidae), native to Europe and northern Africa, was first to arrive in New Zealand. Its arrival is dated to 1944, when queens hibernating in a shipment of aircraft parts made it from Europe at the end of World War II (Thomas, 1960). Despite efforts to eradicate nests, *V. germanica* quickly spread through most of the North Island and parts of the upper South Island. Arrival of the common wasp, *Vespula vulgaris* (L.) (Hymenoptera: Vespidae), which is native to Europe and parts of Asia, is less clear. It may have arrived several times since the early 20th century, but it only became abundant in the 1970s (Donovan, 1984). It spread rapidly and displaced the German wasp, especially in the upper South Island beech forest habitat (Harris, 1991).

Both species are now widespread throughout New Zealand, and in some habitats can be the most common insect encountered (Beggs, 2001; Gardner-Gee and Beggs, 2013). Nest densities can reach 34 nests per hectare (Moller *et al.*, 1990), with inter-annual population dynamics highly responsive to wasp density and to spring weather conditions, similar to the dynamics in the native range (Lester *et al.*, 2017). Wasp biomass in New Zealand is estimated to supersede that of birds and introduced rodents and mustelids combined (Thomas *et al.*, 1990).

Wasps have a detrimental impact on both native ecosystems (Beggs, 2001) and human health (Dymock *et al.*, 1994; Low and Stables, 2006), and they cause economic losses to primary industries (MacIntyre and Hellstrom, 2015). They become particularly abundant in habitats where copious quantities of honey dew are produced by indigenous scale insects (Coelostomidiidae) (Gardner-Gee and Beggs, 2013), and more recently by the exotic giant willow aphid, *Tuberolachnus salignus* Gmelin (Hemiptera: Aphididae), which invaded New Zealand in late 2013.

Control methods applied to date include nest destruction, trapping, baiting, and biological control. A biological control programme against *Vespula* wasps in New Zealand began in the late 1970s (Donovan, 1992). Over 200,000 cocoons of parasitoids of the genus *Sphecophaga* were released between 1985 and 1996 (Beggs *et al.*, 1996; Donovan *et al.*, 1989), but it was evident that they were having difficulty becoming established, and their impact on *Vespula* population dynamics was negligible (Beggs *et al.*, 2008).

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Despite further agents being lined up as potential future candidates, the biocontrol programme was discontinued prematurely following the release of *Sphecophaga* spp.

A discovery of a mite in wasp nests in New Zealand in 2011 sparked new interest in reviving the biocontrol programme. The mite, which was new to science at the time of discovery and has recently been described (Fan *et al*., 2016), was associated with rapid collapse of wasp nests it was found in (B. Brown, personal observation). Further investigation revealed that the mite is not a direct parasite of wasps, but the feeding mode of the mite is disruptive to wasps (B. Brown, personal observation). It is suspected that the mite's feeding is insufficient to cause nests to collapse and there is another mechanism at play. Investigation of pathogens associated with the mites is currently underway.

In 2016 permission was granted to re-introduce new genetic stock of *Sphecophaga*. The rationale in revisiting *Sphecophaga* was that insects collected for the original introductions came from a geographic range that poorly matched the geographic origin of *Vespula* in New Zealand, and that better-matched parasitoids may have a better capacity to evade nest hygiene and have an impact on *Vespula* populations. Recent DNA analysis has determined that the origin of New Zealand's common wasp is in all likelihood south-western UK (Lester *et al*., 2014). A 2016 trip to the south-western UK resulted in the collection of new *Sphecophaga* and, having passed through one generation in containment in New Zealand, this new genetic stock is expected to be released into the wild by late 2017.

We are now entering a new stage in the revived classical biocontrol programme. Three candidate agents are lined up for investigation. Two parasitic flies, the hoverfly *Volucella inanis* (L.) (Diptera: Syrphidae) and the thick-headed fly *Leopoldius* sp. (Diptera: Conopidae), are likely to be joined by the wasp nest beetle, *Metoecus paradoxus* (L.) (Coleoptera: Ripiphoridae). The beetle was listed as a potential candidate in the 1980s programme, but the two flies have not been identified as candidate agents until now.

These three agents target different life stages of wasps and we anticipate their impacts to be complementary: *Volucella* is an ectoparasite of wasp larvae, *Leopoldius* parasitizes adult wasps, and *Metoecus* is an internal parasite of young wasp larvae, and becomes an ectoparasite of later-instar wasp larvae and wasp pupae (Heitmans and Peeters, 1996). *Volucella* and *Metoecus* have already been detected at potential collection sites in the UK, which we intend to visit in August/September 2017. *Volucella* was found in high abundance in the 2016 trips to collect *Sphecophaga* from the UK. A fair bit is known about its biology, and it is therefore prioritised as top of the candidate list. *Metoecus* has a complex life cycle (Heitmans and Peeters, 1996), which could make it quite difficult to perform tests on in containment, and therefore currently it sits at the bottom of the priority list.

Some of these candidate agents can attack other vespids, which is considered an advantage: the entire family Vespulidae is absent from New Zealand's indigenous fauna, and any potential future invaders could thus be targeted by these oligophagous agents.

Acknowledgements: This programme would not have been possible if it wasn't for joint widespread support from the Ministry for Primary industries' Sustainable Farming Fund, and a large number of regional and district councils, primary industry sectors and community groups, all united in a desire to find a long-term solution to wasps.

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Poster 32: The Rich Tapestry of Biological Control Targets and Agents in Sweetpotato Production Systems of Papua New Guinea

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We introduce our ongoing work in the sweetpotato production systems of Papua New Guinea (PNG) where cash-cropping is developing from subsistence farming. Here it is common for newly planted ‘gardens’ to be established close to, or even immediately adjacent to, old ‘gardens’ in which multiple successive sweetpotato crops have been grown. Increased pest and disease densities in old ‘gardens’ (along with nutrient depletion) cause marked decreases in vigour and health of crops (Fig. P32.1). These biotic threats may readily more to nearby new ‘gardens’. Surveys of growers have shown high levels of pest and disease impact on sweetpotato (Gurr *et al.*, 2016). Weevils, gall mite and scab are the top three major crop protection issues for farmers (Fig. P32.2) in both new and old ‘gardens’.

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Fig. P32.1. Contrasting vigour of sweetpotato crops in old ‘gardens’ (left) and new ‘gardens’ (right). (Photo: G.M. Gurr).

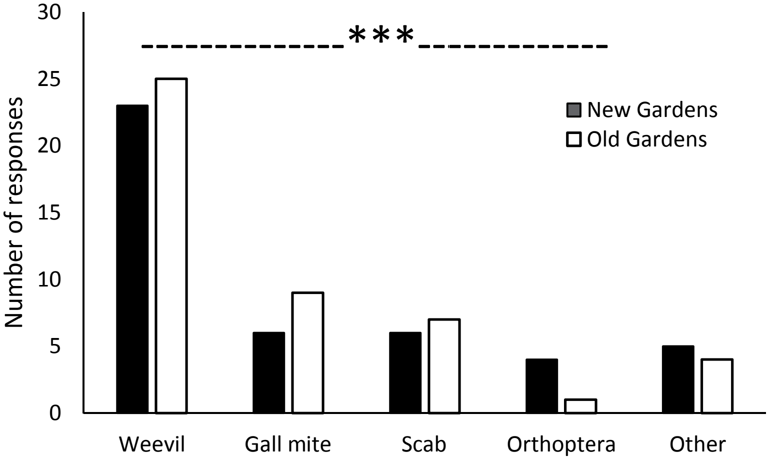

Fig. P32.2. Major plant protection issues cited in the top three concerns by farmers’ for pest and disease problems. (χ^2 tests compared pest types within each garden age: new gardens, priority is used by times been listed without giving any points. Weevil: χ^2 =16.448, df =4, p=0.002; old gardens, χ^2=23.836, df =4, p<0.001).

Despite growers reporting use of a wide range of plant protection methods including biological control with chickens and pigs, a large majority use no active intervention (Fig. P32.3). Our work is evaluating the wider potential for other forms of biological control intervention to complement traditional practices such as the use of domesticated vertebrates to consume pests and crop residues. Field surveys in PNG as well as in Australia are being used to explore the diversity of natural enemies, whilst laboratory and field studies are comparing candidate entomopathogens, e.g., *Metarhizium* spp. (Clavicipitaceae) and *Pasteuria* spp. (Pasteuriaceae). Complementary work is exploring the potential benefits of using native and naturalised plant species as barrier plants around new 'gardens' to reduce immigration of pests, especially weevils, from old 'gardens'. Among these plants are *Tephrosia* spp. (Fabaceae) (Fig. P32.4) that are known to have biocidal/ repellent properties (Belmain *et al.*, 2012) and may also provide flora resources to natural enemies such as parasitoids.

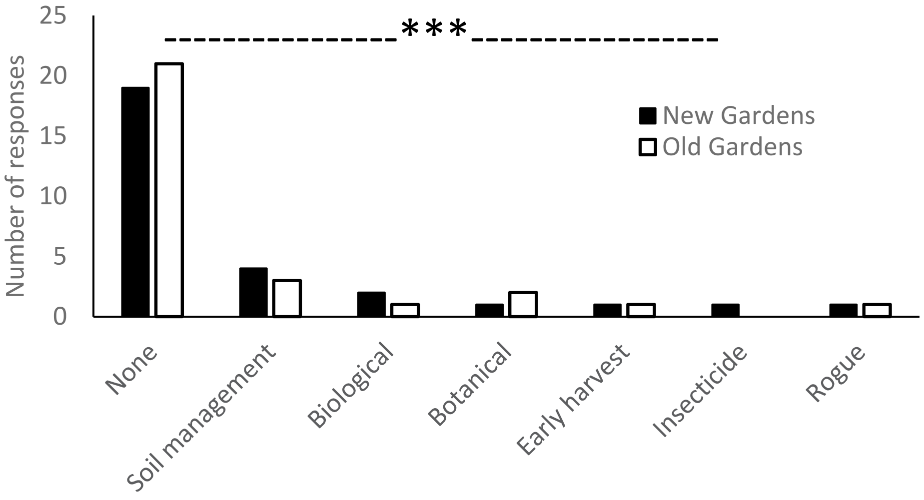

Fig. P32.3. Reported actions taken to control pests on sweetpotato crops. (χ^2 tests compared management approaches within each garden age: new gardens, $\chi^2 = 41.989$, df =6, $p<0.001$; old gardens, $\chi^2 = 52.738$, df =6, $p<0.001$.).

Fig. P32.4. Uncultivated *Tephrosia* sp. in the Highlands of Papua New Guinea; a potential barrier to pest movement and nectar source for natural enemies. (Photo: G.M. Gurr).

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Poster 33: *Acerophagus papayae* Noyes and Schauff (Hymenoptera: Encyrtidae) as a Biocontrol Agent of *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) in Barbados

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Paracoccus marginatus Williams and Granara de Willink (Hemiptera: Pseudococcidae) is a polyphagous insect that attacks different crops and ornamental plants (Williams and Granara, 1992; Walker *et al.*, 2011). Although *P. marginatus* has been recorded in Central and South America, Mexico, Florida and the Caribbean islands, only in Florida and the Caribbean the pest is considered of economic importance (Pantoja *et al.*, 2002). Host plants of economic importance include: papaya, citrus, tomato, bean, potato, cotton, mango, among others.

The parasitoid *Acerophagus papayae* Noyes and Schauff (Hemiptera: Pseudococcidae) has been reported as an efficient biocontrol agent of *P. marginatus* (Noyes and Schauff, 2003; Nisha and Kennedy, 2016). The parasitoid specie was already present at the field and it was observed attacking the *P. marginatus* colonies. The biology of the parasitoid and *P. marginatus* was studied and it was evaluated the influence of three host plants: Papaya, *Carica papae* L. (Caricaceae), Bean, *Phaseolus vulgaris* L. (Fabaceae) and Sea Island Cotton, *Gossypium barbadense* L. (Malvaceae) on the development of the pest and parasitoid. For *A. papayae* it was evaluated the preferred nymphal stage and the percentage of parasitims on nymphs reared in different hosts.

The experiment was carried out in the facilities of Ministry of Agriculture and Rural Development of Barbados. The biology of *P. marginatus* was carried out in greenhouse and laboratory conditions (temperature of 26 ± 2 °C; RH 70 ± 10 % and photophase 14 h). The biology of *A. papayae* was studied only in laboratory condition. Ten plants of each host were infested, using 70 nymphs/plant. The observations were done daily until the insects reached adult stage. The parameters evaluated were: a) Immature phase: longevity of female and male and viability, b) Mature phase: female and male longevity, viability and

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number of eggs by female, sex proportion, c) Egg phase: period of emergence of the nymphs and eggs viability.

In order to evaluate the host preferance by *A. papayae*, it was carried out the study of the biology of the parasitoid. Seventy nymphs of 1st, 2nd and 3rd instars were separated, placing them in plants of each host, having 10 replications. Each plant was placed in a cage (50 x 50 x 50 cm). Ten couples of the parasitoids were released in each cage and removed after 24 hours. To determine the number of nymphs parasitized by each female, 30 nymphs were maintained in leaves and offered to a parasitoid couple for 24 hours. The observation of the material was done daily. To determinate other biological parameters, 70 nymphs from the 3rd instar were separated and exposed to 10 parasitoids for 24 hours. The biological parameters evaluated were: a) Adult phase: male and female longevity with and without parasitic activity, parasitism and superparasitism frequency and sexual proportion. b) Lifespan: duration.

The statistical analysis was conducted with ANOVA, t-test of Student (LSD), 5% probability with 10 replications.

When evaluating the different hosts, there was a statistically significant difference showing Papaya as the most favorable host for the development of *P. marginatus* (Table P33.1). Our results agree with those of Williams and Granara-de-Willink (1992), Meyerdirk and Kauffman (2001), Nisha and Kennedy (2016), who reported papaya as the primary host, while bean and cotton were registered as secondary hosts. In the experiment, a statistically significant difference was observed in the number of eggs per female when *P. marginatus* was mantained in greenhouse conditions. The highest number of eggs was registered in papaya with a mean of 436.3 eggs (Table P33.2). The same results were observed at laboratory conditions, having a mean of 334.6 eggs per female when using Papaya as a host. The less favarable host was the Sea Island Cotton in both, green house and laboratory condition. Since the duration of the immature stage and viability were affected, the effect on reproduction was probably a consequence of this.

Table P33.1. Preference (%) of *Acerophagus papayae* by nymphs of different instars of *Paracoccus marginatus* in papaya under laboratory conditions, Barbados, 2003.

Nymphal Instar	Mean ± SE
1st	04.4 ± 0.63 d
2nd	17.7± 1.108 b
3rd	80.4 ±2.83 a
C.V. (%)	21.33
DMS a 5%	5.86

* Means followed by the same letter do not differ from each other by Student's T test (LSD), at 5% probability level.

Table P33.2. Number of eggs (mean ±SE) per female *Paracoccus marginatus* maintained in three hosts in greenhouse and under laboratory conditions, Barbados, 2003.

	Number of eggs/ female (Mean ±SE)		
	Papaya	**Bean**	**Cotton**
Green House	436.3 ±20,79 a	278.1 ±24.92 a	105.2 ±08.11 a
Laboratory	334.6 ±23.52 b	208.0 ±16.28 b	68.70 ±05.87 b
C.V. (%)	24.1		
DMS a 5%	51.8		

* Means followed by the same letter do not differ from each other by Student's T test (LSD), at 5% probability level.

The 3rd nymphal instar was observed to be the preferred instar by *A. papayae*, showing 80.4 % of parasitism in nymphs reared in Papaya. The less favorable one was the first instar having 4.4 % of parasitism (Table P33.2). This information will be an important factor when mass producing *A. papayae* to be used in biological control programmes.

The results showed that the percentage of parasitism was also affected when using different hosts. The most favorable one was papaya showing 86.1 % of parasitims, when compare with Sea Island Cotton, which was the less favorable host having 49.1 % of parasitism (Table P33.3). The results show the potential of the use of papaya as a host when rearing *A. papayae* for its use in biological control programmes.

Table P33.3. % of parasitism (mean ±SE) in 24 h of *Acerophagus papayae* on *Paracoccus marginatus* nymphs kept in different hosts under laboratory conditions, Barbados, 2003.

Host Plants	**Parasitism (%) Mean ±SE**
Papaya	86.1 ±1.70 a
Bean	68.9 ±2.16 b
Cotton	49.1 ±2.31 c
C.V. (%)	10.81
DMS a 5%	6.91

* Means followed by the same letter do not differ from each other, by Student's T test (LSD), at 5% probability level.

It was verified the potential of *Acerophagus papayae* as an efficient biocontrol agent to be used in biological control programmes of *Paracoccus marginatus* in the Caribbean.

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Poster 34: Economic Impact of Biological Control of Mango-infesting Fruit Flies: A Case Study of Kenya

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The impact of the parasitoids *Fopius arisanus (*Sonan) (Hymenoptera: Braconidae) and *Diachasmimorpha longicaudata (*Ashmead) (Hymenoptera: Braconidae) for fruit fly suppression was evaluated in combination with other management strategies within a framework of integrated pest management (IPM), in comparison to farmers' practices as a control group, in Meru County, Kenya. Our study findings showed that parasitoid release in combination with other strategies had a significant impact on mango revenues compared with the control group. While the average expenditure on pesticides decreased across all mango farmer households, the reduction was comparable between the treated and control farms. Further, significant decrease in mango damage due to fruit fly infestations among all farmers using the different IPM combinations was observed. The use of parasitoid for the invasive fruit fly *Bactrocera. dorsalis* (Hendel) (Diptera: Tephritidae)with a holistic IPM strategy is discussed in the light of the findings of this study.

In Kenya, mango enterprise contributes significantly to the agricultural Gross Domestic Product (GDP) and foreign exchange earnings. Mango production in the country however is stil below its potential, due to various constraints, paticularly high insect pests' infestation, chiefly the tephritid fruit flies (e.g., *B. dorsalis*, *Ceratitis cosyra (*Walker) (Diptera: Tephritidae), etc.). In addition to reducing yield, quality and value of the mango, fruit fly-infested produce is quarantined from lucrative markets abroad. In Africa, icipe has spearheaded development and implementation of an integrated pest management strategy for managing fruits flies as an alternative to the oftenly used harmful and expensive synthetic pesticides, with a goal of reducing mango losses, lower the cost of production, increase producer income and improve market access and processing (Ekesi *et al.*, 2011). The IPM strategy consisted in five components, i.e (1) spot application of food bait, **fb**, (2) male annihilation technique, **mat**, (3) use of biopesticide, **biop**, (4) releases of parasitoids, **p** and (5) use of orchard sanitation,**os**. This study aims at evaluating the impact of application of various combinations of the five components on pesticide expenditure, mango fruit yield loss and profit.

Our study utilized data collected before and after the IPM intervention in treatment and a control areas in Meru County (Kenya). Two-stage sampling framework was adopted, where four sub-counties where mango production is predominant in the county was selected (namely Central Imenti, North Imenti, and South Imenti (treatment areas), and Tigania West (control area)). In the second stage, 153 mango farmers were randomly

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selected from each sub-county to receive certain combination of the five components, while 179 mango farming households were selected for interviews in the control area. A total of 1,223 mango farmers were successfully interviewed during the first round of the survey (November and December 2013), while 1,122 farmers was revisited between May and June 2014. A final sample of 828 households including 694 IPM users and 134 control farmers was used for analysis. A structured questionnaire was used to capture important variables related to mango production and other contextual data. We adopted difference-in-difference (DiD) model to estimate the difference between the observed mean outcomes for the treatment and control groups before and after the IPM intervention.

We evaluated the impact of different combination of IPM components on mango income, mango losses due to fruit fly infestation and expenditure on synthetic pesticides. Net income from mango was computed as the total revenue less the variable costs per unit land (acre). Mango losses were calaculated as the proportion of mango damage due to fruit fly infestation out of the total mango crop produced (De Groote, 2002). Mango yield included sales and home consumption, based on farmer's estimates and divided with area planted to mango. Pesticide expenditure included the cost of purchasing pesticides to control various pests and diseases per unit land during a specific season.

Table P34.1. DiD models for mango fruit fly IPM strategy effects on income, pesticide expenditure and mango loss.

	Mangoes net income (KSh/acre) (1)	**Total cost of pesticides (KSh/acre) (2)**	**Mangoes damaged by fruit fly (percent of total production) (3)**
Follow up (θ)	-3,825.05	-2,632.85***	10.43***
	(5,651.2)	(476.6)	(1.94)
Posbiop*follow up (Γ_1)	9,116.48	-584.17	-27.45***
	(11,505.9)	(845.5)	(2.83)
Posfb*follow up (Γ_2)	72,097.30***	901.22	-29.29***
	(24,724.7)	(857.1)	(2.67)
Posfbbiop*follow up (Γ_3)	38,898.18**	507.2	-35.48***
	(19,417.2)	(1,476.1)	(3.00)
Posmat*follow up (Γ_4)	28,789.32**	-111.01	-29.18***
	(12,163.0)	(934.2)	(2.65)
Posmatbiop*follow up (Γ_5)	34,705.85***	672.73	-32.90***
	(9,780.7)	(684.0)	(2.49)
Posmatbiopfb*follow up (Γ_6)	42,191.18**	-306.8	-26.99***
	(17,953.6)	(896.9)	(2.66)
Posmatfb*follow up (Γ_7)	69,969.48**	579.11	-28.35***
	(27,682.2)	(902.0)	(2.58)
Pos*follow up (Γ_8)	22,165.91	-215.8	-24.00***
	(15,533.8)	(968.3)	(2.85)
Number of observations	1,656	1,656	1,656
F	9.14***	9.12***	87.53***
R-squared	0.09	0.10	0.52

Note: Robust standard errors in parenthesis. The abbreviations for the IPM interventions are as follows: **P**=parasitoids release; **os**=orchard sanitation; **fb**=food bait; **biop**=biopesticides; **mat**=male annihilation technique * p<0.10, ** p<0.05, *** p<0.01. household and farm characteristics are included in the analysis but not shown in the table. 1 acre= 0.4 ha; The exchange rate at the time of the survey was approximately 85 Kenyan Shillings (KSh)/US $. sh is abbreviation for Kenya shillings; The estimation also includes individual treatment variables. *Source*: - Muriithi *et al.* (2016).

Descriptive statistics shows that combination of parasitoid, orchard sanitation and food bait and/or male annihilation technique, results to positive and significant change in mango revenue. Similarly, all combinations result in significant reduction in mangoes damaged by fruit flies.

The empirical analysis showed that, for some treatments, net income increased between the two-survey rounds compared with the control group (Table P34.1). The change in net income was higher for mango producers who applied any of the following six combinations: *posfb, posfbbiop, posmat, posmatbiop, posmatbiopfb and posmatfb*. Parasitoid and orchard sanitation alonewere not be sufficient to positively impact mango revenues. With respect to cost of pesticides, there was significant decrease (Ksh. 2,630 per acre), independent of the IPM strategy. However, there was no impact observed when we consider specific IPM combination, suggesting that farmers could be using pesticides to control other pests and diseases other than fruit flies. In terms of mango damaged by fruit fly, significant decrease was reported across all the IPM combination (column 3), including ***pos*** combination, compared with the change observed from control group, before and after the intervention.

Comparisons before and after the interventions showed that, on average, mango net income increased by 48% across all combinations of treatments, with treatments *posmatfb* and *posfb* reporting highest profit margin while *pos* reported the least; this implies that biological control alone may not be sufficient for the suppression of the *B. dorsalis*. Regardless of the treatments combination, expenditure on pesticides was reduced by an average of 45%, while mango losses due to fruit fly infestation was reduced by 17%. The empirical analysis showed positive and significant increase in net income for most treatments compared to the control group, with the highest impact reported from *posfb*, while *posmat* showed the least impact. With respect to mango losses, there was a significant decrease across households adopting different treatments, with the largest decrease observed among farmers combining parasitoids, food bait and biopesticide (*posfbbiop*). Our study recommends the need to combine affordable and easy to apply (and maintain) IPM strategies, for instance, biological control (parasitoids), cultural control (orchard sanitation) and minimal (and affordable) baiting techniques using food bait that could yield significant impact on mango fruit fly control.

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Poster 35: 'Nothing Kills Insects', or How Public and Farmer Perceptions Affect Success Rates of Biological Control

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Though regularly overlooked, social science is of paramount importance to the practice of biological control. On the one hand, cultural trends have major influences on local, national and global adoption rates of certain technologies or practices, and public perceptions are not always favorable towards biological control practices. On the other hand, knowledge, attitudes and beliefs of farmers themselves greatly influence field-level adoption rates and subsequent diffusion of biocontrol technologies. In this study, we conduct a critical analysis of the extent to which 'social science' aspects have been taken into account in biological control research worldwide, and have a closer look at (historic trends in) public and farmer attitudes towards particular biological control technologies and approaches.

First, we conducted a retrospective analysis of the degree to which social science facets have been incorporated into biological control research over the past 25 years. We ran an ISI Web of Knowledge search for insect biological control studies that deliberately took into account social science aspects. Covering nearly 12,000 journals, our search was restricted to papers that were published over the time period 1990-2016. Within the resulting literature base of 11,732 manuscripts, a total of 161 studies (or 1.4%) were found in which reference was made to farmers, stakeholders or the general public. Even fewer papers (i.e., a total of 28) covered aspects such as knowledge transfer and technology diffusion. As little as four publications made reference to gender aspects, and no studies were found in which explicit emphasis was given to youth or young farmers.

Next, we made use of powerful text-mining tools and social-media analytics, to assess temporal and geographic trends in cultural visibility of the topic of 'biological pest control' and particular globally-important case studies. As systematic surveys of public perceptions and attitudes have rarely been conducted, we opted for the use of Google-based search engines to obtain quantitative metrics of global public interest in biological control. To investigate cultural trends quantitatively, we employed a new data collection and analysis technique, i.e. 'culturomics'. Culturomics provides text-mining tools and social-media analytics to classify, quantify, and visualize cultural values on a range of topics. First, we used Google Ngram Viewer as an online search engine to look for coverage of different terms in sources printed between 1950 and 2008 (covering a corpus of >15 million digitized books). Next, we conducted time series analyses through Google Trends in online search data from 2004 to 2016 to investigate whether (a) public interests in 'biological pest

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control' has declined over time and (ii) online interest patterns for particular topics related to pest management differ between continents.

Google Ngrams depict how the search string 'biological control' was most frequently recorded in digitized books in 1993, after which it gradually dropped until 2001 and then steeply declined. On the other hand, interest in 'ecological safety' steadily built up from the early 1980s until 1997-98, and then dropped from 2002 onwards. For a select set of (classical) biological control cases, Google Ngrams show highly variable and shifting temporal patterns in cultural visibility over a 1950-2008 time window. As a positive note, successful biocontrol cases such as the cottony cushion scale x Vedalia beetle (1888) or the *Anagyrus lopezi* Desantis (Hymenoptera: Encyrtidae) x cassava mealybug biocontrol programs (mid-1980s) continue to be featured. The Vedalia beetle case continues to be celebrated, although its target pest nearly disappeared from California's citrus orchards, in similar ways as e.g., extinct North American birds. On the other hand, public interest in either success story has gradually dropped from 1974 and 1996 onward. Surprisingly, similar drops in cultural visibility were recorded after 1995, 1985 and 1996 for *Encarsia formosa* Gahan (Hymeoptera: Aphelinidae), *Phytoseiulus persimilis* Athias-Henriot (Megostigmata: Phytoseiidae) or *Trichogramma* spp. (Hymenoptera: Trichogrammatidae); flag-bearers for augmentation biological control that continue to be used extensively in greenhouse and open-field cultivation. Lastly, the rare cases in which introduced agents caused un-intended ecological side-effects (e.g., cane toad, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) currently receive comparatively high, if not escalating, cultural visibility. Hence, at least up till 2008, cultural saliency of the world's reliable, effective natural enemies was dramatically lower than that of the handful of cases where (classical) biological control failed.

Next, Google Trends analyses were run for several topics related to crop protection and invasive species management. Google Trends is regularly used to track levels of public interest and awareness at a global or country-specific level. We assessed trends in Google search volume for the topic 'biological pest control' over a 2004–2016 time frame, recording proportionate traffic to the search volume of all keywords submitted in Google, scaled on a range of 0 to 100. We corrected for this shifting baseline in Google search data by dividing search volume for 'biological pest control' by that of benchmark keywords "computer" and "life". Seasonal Mann-Kendall tests were performed on corrected search data, using package Kendall in R. When using "life" as the benchmark, a statistically significant downward trend was found (tau= -0.317, p< 0.001). Also, when examining country-specific patterns, 'biological pest control' represents a substantial part of Google search volume in countries such as Costa Rica, Colombia, New Zealand or Algeria.

Internet search analysis thus provided a powerful, systematic and data-driven means to gauge (online) public interest in biological control. While 'biological control' clearly is not a "trendy" search-term and faces a stagnating or even downward trend in Google search volume and Ngram string frequency, similar patterns are reported for other environmental topics. Despite their drawbacks and limitations, culturomics could be used to strategically reposition biological control, e.g., by a) picking 'winners' for targeted communication campaigns, based upon the extent to which particular species resonate with the general public; b) assessing cultural impacts of specific invasive species, and eventual biocontrol interventions; and c) identifying geographies where biological control continues to have a soundboard with online communities and interest groups.

Aside from examining (online) public interest and the degree of attention that's been paid to 'social science' aspects in biological control publications, it is crucial to take into account the ultimate adopters and have a closer look at patterns in farmer knowledge.

Anthropologists and sociologists have realized, since the early 1990s, that the vast majority of farmers is largely unaware and un-informed about natural pest control. Even (smallholder) growers who maintain a close bond with nature were either entirely unaware of natural pest control, or expressed doubt about the actual value of these services on their farm. We examine a number of case studies in the developing-world tropics, covering farmer knowledge of weaver ants in Vietnamese citrus orchards, mealybug parasitoids in Asian cassava fields, and lepidopteran pests in Central American maize x bean systems. Overall, we conclude that local (smallholder) farmers have very limited understanding of the entomofauna in their fields, and only occasionally realize the beneficial role of resident parasitoids, predators or (even less) entomopathogens. With those farmers, the promotion of (knowledge-intensive) technologies such as biological control likely will be particularly challenging and if not impossible.

In conclusion, biological control has rapidly lost cultural saliency and biological control failures have come to receive disproportionately high public attention since the early 2000s. Sustained (online) public interest though does exist in multiple vestiges around the world, particularly in the developing world. Though a number of high-profile biological control programs are underway (e.g., cassava mealybug biocontrol in Asia; biocontrol of parasitic flies on Darwin's finches), these so far receive relatively minor attention. Also, after decades of promoting IPM and biocontrol across the developing world, we realize that the bulk of (resource-poor, illiterate) smallholders simply has no understanding of beneficial organisms within their fields (or, for that matter, of ways to enhance their abundance or efficacy). Now more than ever, biological control is at a crossroads and cross-disciplinary science is promptly needed to help bolster adoption rates, counter-act surging insecticide use, and restore public trust in one of nature's prime services.

Poster 36: Evolution and Potential Non-target Effect of the Introduced Biological Control Agent *Cryptolaemus montrouzieri*

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Cryptolaemus montrouzieri Mulsant (Coleoptera: Coccinellidae), the mealybug destroyer, is a predatory ladybird of Australian origin and has been introduced worldwide to control mealybugs. We first found different performances of this introduced ladybird from different areas. Then, we detected strong genetic differentiation and local adaptation among the worldwide distributed populations by analyses of mitochondrial genomes, microsatellite and reduced-representation genomes. These genetic markers also suggested a history of population admixture, which were further confirmed in a lab-simulated experiment. Finally, the test of diet ranges and analysis of adaptation to diet shifts suggested potential non-target effect of this introduced ladybird.

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Poster 37: Bioassay and Scanning Electron Microscopic Observations Reveal High Virulence of Entomopathogenic Fungus, on the Onion Maggot

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The onion maggot, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae), is a devastating pest of spring-grown onions, *Allium cepa* L., and chinese chive, *A. tuberosum* Rottler ex Spreng (Amaryllidaceae) (Eckenrode *et al.*, 1975; Eckenrode and Nyrop, 1986; Nault *et al.*, 2006; Song *et al.*, 2007), in northern temperate zones, especially in northwestern North America and northern China (Toepfer *et al.*, 2014). The entomopathogenic fungus, *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Clavicipitaceae), has been shown to be a potential biological control agent against onion maggots (Poprawski *et al.*, 1985; Davidson and Chandler, 2005).

Although scanning electron microscopy (SEM) has frequently been used to evaluate the infection process of entomopathogenic fungi in many insect hosts (Vieira *et al.*, 2013; Wu *et al.*, 2014), the technique has not been used on onion maggots or any of their closely related species. Recent studies have focused on the screening and mass production of entomopathogenic fungi (Tumuhaise *et al.*, 2015). In addition, previous studies have postulated that the dense microchaetae actually enabled the conidia to attach, and that the abdominal membrane was the major site of conidial attachment (Wang *et al.*, 2005, 2010, 2011). To better understand the major site of conidial attachment and penetration and the factors involved in the process of *B. bassiana* infection of onion maggot adults, we studied the fungal infection process of the virulent isolate XJWLMQ-32 using SEM. We evaluated whether abdominal applications are the best strategy for optimal infection. Our study also focused on evaluating the efficacy of *B. bassiana* isolates against onion maggot adults. This information will enhance our understanding of the infectivity of *B. bassiana* in onion maggot and will help to determine the possibility of its use in biocontrol programs.

When flies were dipped in 1 x 10^8 conidia/ml conidia suspensions and then kept in the incubator (22 ±1 °C, 70 ±5% RH), scanning electron microscope observations revealed that, at 2 h, the majority of adhering *B. bassiana* conidia were attached to either the wing surface or the interstitial area between the macrochaetae on the thorax and abdomen of the onion maggot adults (Fig. P37.1). Germ tubes were being produced and had oriented toward the cuticle by 18 h. Penetration of the insect cuticle had occurred by 36 h, and by 48 h, germ tubes had completely penetrated the cuticle. Fungal mycelia had emerged from the insect body and were proliferating after 72 h (Fig. P37.2). The superficial area and structure of the wings and macrochaetae may facilitate the attachment of conidia and enable effective

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penetration. The susceptibility of adults to 12 isolates, at a concentration of 1 x 10^7 conidia/ml, was tested in laboratory experiments. Eight of the more potent strains caused in excess of 85% adult mortality 8 d post inoculation, while the median lethal time (LT_{50}) of these strains was <6 d. The virulence of the more effective strains was further tested, and the median lethal concentrations (LC_{50}) were calculated by exposing adults to doses ranging from 10^3–10^7 conidia/ml. The lowest LC_{50} value, found in the isolate JWLMQ-32, for the adults was 3.87 x 10^3 conidia/ml. These results demonstrate that some *B. bassiana* strains are highly virulent to onion maggot adults and should be considered as potential biocontrol agents against the adult flies.

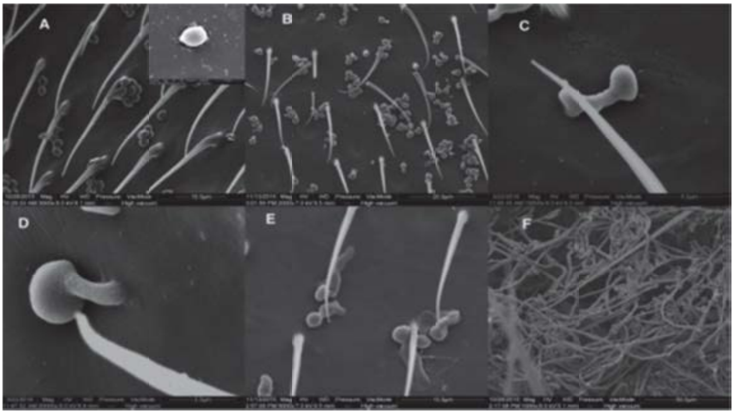

Fig. P37.1. Germination and penetration of B. bassiana strain XJWLMQ-32 conidia on the cuticle of *Delia antiqua* adults: **A)** conidia adhering to the cuticle of *Delia antiqua* and secretion at the interface of conidia and cuticle at 2h; **B)** generation of conidial germ tube and orientation toward the cuticle at 18hr; **C)** conidial germ tube and appressorium generation at 24 h; **D)** germ tube penetration of the cuticle at 48 hr; **E)** fungal hyphae growing on the cuticle; **F)** conidia emerging from the dead adult at 72h.

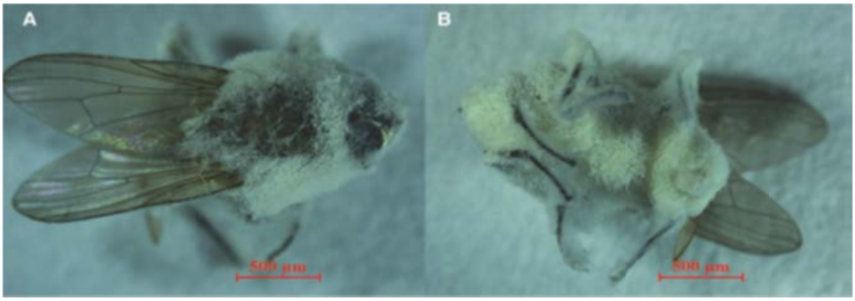

Fig. P37.2. Conidia emerging from all surfaces of a dead adult. **A)** view of the thorax of the dead adult; **B)** view of the abdomen of the dead adult.

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Poster 38: Cattle Fever Tick, *Rhipicephalus annulatus* (Acari: Ixodidae), and the Quest for Discovery of Its Natural Enemies in the Balkan Region

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Rhipicephalus annulatus Say (Ixodida: Ixodidae), the Cattle Fever Tick (CFT), is a hard tick native to Mediterranean region with several hosts such as cattle and white tailed deer. It transmits two lethal diseases, piroplasmosis and babesiosis. It is an invasive species in US, with a significant impact on cattle production especially in Texas. Extensive use of acaricides resulted in resistance. Special attention is given to the Balkan region for discovery of natural enemies since molecular analysis of CFT showed that the Texas population is similar to those of Bulgaria and Romania. Biological control of CFT is the main objective of this study. There are few examples of successful classical biological control of livestock pests such as blood-feeding bush flies, *Musca vetustissima* Walker, and buffalo flies, *Haematobia irritans* (L.) (Diptera: Muscidae) (Waterhouse, 1974). Attempts for possible biological control have been focused on the use of known parasitic wasps of the genera *Ixodiphagous* (Hymenoptera: Encyrtidae). There are seven recognized species: *Ixodiphagus texanus* Howard, *Ixodiphagus hookeri* (Howard), *Ixodiphagus mysorensis* Mani, *Ixodiphagus hirtus* Nikolskaya, *Ixodiphagus theilerae* (Fielder), *Ixodiphagus biroi* Erdos, and *Ixodiphagus sagarensis* (Geevarghese). These parasitic wasps parasitize ticks belonging to the genera *Ornithodoros*, *Amblyomma*, *Dermacentor*, *Hyalomma*, *Haemaphysalis*, *Ixodes*, and *Rhipicephalus*. These parasitic wasps seem to have 1-2 generations per year (Hu *et al*., 1998).

Nematodes have also been investigated as biological control agents for ticks (Samish and Glazer, 1991; Samish and Rehacek, 1999; Samish *et al*., 2004). Most of the research has involved use of commercially available entomopathogenic nematodes of the families Heterorhabditidae and Steinernematidae. The only free-living stage of the nematode, the third/infective juvenile, actively locates and enters the host via natural openings, and then

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releases symbiotic bacteria that kill the host within 24–72 h. The nematodes then multiply within the host cadaver, and by 6–18 days post infection, thousands of infective juveniles are released into the environment. However, currently available entomopathogenic nematodes such as *Steinernama carpocapse*, can affect and kill CFT, but do not reproduce (Samish *et al.*, 2004, Molina-Ochoa *et al.*, 2009).

Extensive travelling and cooperation with various institutions and small family farms which are not using any or have very restricted use of acaricide products in Greece, Turkey, Albania and Bulgaria resulted in the creation of a wide network of potential tick exposure sites which we hope to result in location and development of specialized parasitoids or entomopathogenic nematodes for biological control of CFT.

Materials and Methods: In each of three locations in Greece and Bulgaria, two, six to nine months old calves were used for exposure of *Rhipicephalus annulatus* to its natural enemies. Two thousand laboratory reared tick larvae were transferred to the back of the calves which were kept in a cattle chute. They were given enough time to seek the location of their preference on the calves where they would chose to have their blood meal. The calves were then released from the chute into a large pen or into nature for feeding and the exposure of ticks to their possible natural enemies for 25 days. After this they would be returned to a smaller, approximately 5 m x 5 m pen in which the ground would be cleaned beforehand to be able to see the falling engorged ticks easier. Claves would be fed in this pen and the ground of the pen would be twice checked for the presence of fallen, engorged ticks. These were collected and transferred to a vial covered with a piece of cotton for air exchange. All the vials were kept in an incubator at 27°C, 80% RH and 12 hours light and 12 hours darkness for emergence of possible parasitoids or entomopathogens.

Soils samples from resting places of cattle, sheep and goats from various regions of the above mentioned countries were collected. Wax moth, *Galleria mellonella* (Fabricius) (Lepidoptera: Pyralidae), larvae were exposed these soils to detect nematodes which could attack the ticks feeding on the cattle, sheep and goat.

Tick eggs were exposed to possible egg parasitoids and this was documented by using a Brinno camera taking pictures every 1 sec. The same method was used to find out whether any predators would feed on seeking larvae.

Reared parasitoids or nematodes then will be used for host specificity tests to determine host specificity and whether their uses for biological control of cattle fever tick could be recommended.

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Poster 39: Can Pesticide-treated Nets be a Tool in IPM of Horticultural Crops?

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Insecticide-Treated Nets (ITNs), widely used for controlling mosquitoes which vector human diseases (Hougard *et al.*, 2002; WHO, 2014), are now under focus for use in agricultural systems (Martin *et al.*, 2010; Dáder *et al.*, 2014, 2015). Depending on the insecticide, the hole size and the way they are produced, these nets can target different pests and are interesting options for use in integrated pest management programmes (IPM).

The efficacy of ITNs is very much linked to the size and geometry of the hole and to the kind of incorporated pesticide and they can act both as a physical and chemical barriers by having direct or repellency effects (Martin *et al.*, 2014). The yarns can have different diameters and be knitted in different patterns determining different hole sizes, which must be big enough to allow proper ventilation and to prevent pests passing through or to permit them acquiring sufficient pesticide while crossing the net, so that the number of living pest individuals in the crop is strongly reduced. The pesticides commonly used in these nets are pyrethroids because they are relatively safe for human health and domestic handling and exhibit a rapid knockdown action and high insecticidal potency at low dosages (Zaim *et al.*, 2000; WHO, 2015). In the modern Long Lasting Insecticide-Treated Nets (LLITNs), pesticides are incorporated in the process of making the yarns in the factory, slowing down considerably the degradation process compared to traditional ITNs, which are only externally treated with them (Martin *et al.*, 2007).

As the information available on the compatibility of pesticide-treated nets with beneficial fauna is negligible, we have tested the compatibility of an experimental bifenthrin LLITN (3.6 g/Kg initial content) under laboratory, semi-field and commercial greenhouse conditions, with *Amblyseius swirskii* (Athias-Henriot) (Megostigmata: Phytoseiidae) and *Eretmocerus mundus* (Mercet) (Hymenoptera: Aphelinidae), important natural enemies of whiteflies and thrips in horticultural crops in Spain (Robledo *et al.*, 2009).

In the laboratory, the treated-net was very deleterious, due to residual contact exposure, to adults of both natural enemies, after 72-h. However, in choice tests with Y-tubes (Fig.

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P39.1), both natural enemies were neither attracted nor repelled by the treated-net and no short-term mortality was detected in individuals that had crossed it.

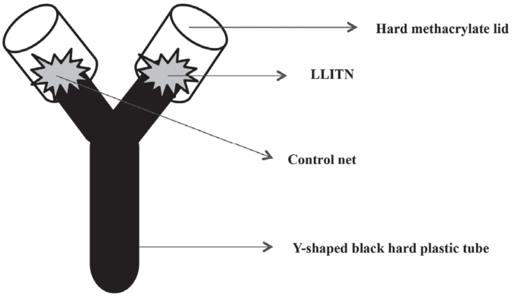

Fig. P39.1. Schematic graphic of the Y-shaped tube used in the choice tests.

Semi-field trails (24.85 ± 0.48 °C, 56.81 ± 1.66% RH and 15-h light natural photoperiod) were performed with tomato plants infected with *B. tabaci* nymphs, enclosed in 60 cm high cages with the control or bifenthrin-treated net placed in one of the sides. *E. mundus* adults were allowed to parasitize the pest for 72 h and neither an effect on the pest (nymphs alive, dead and parasitized) nor on the natural enemy (number emerged and dead during emergence) was detected.

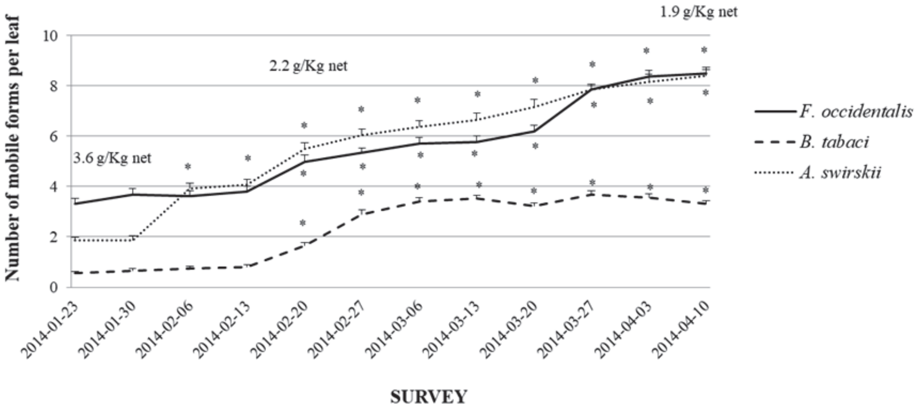

Fig. P39.2. Field assay in commercial cucumber greenhouses of Southeastern Spain. Development of the pest and natural enemy population levels under the bifenthrin-treated net (initial concentration: 3.6 g bifenthrin/Kg net) placed in the laterals and pesticide content in the net along the time.

In field trials performed in January 2014 in commercial pepper greenhouses in South-Eastern Spain (Almeria province) (average environmental conditions outside: 12.32 ± 0.34°C temperature and 67.27 ± 2% relative humidity; inside: temperatures reached 25°C from 11 am to 3-4 pm and decreased up to 7-8 degrees at night), the LLITN proved to be compatible with *A. swirskii* (Fig. P39.2). The pests, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) and *Bemisia tabaci* (Gennadius) (Hemipters: Aylerodidae), and the natural enemy population levels increased with the decrease of bifenthrin content in the net (g/Kg): 3.6 initial- 2.2 after 1-month exposure and 1.9 after 3-months exposure when the trial ended, due to the high light intensity and UV radiation typical in the Mediterraneam area (Fernández *et al.*, 2017).

Therefore the bifenthrin LLITN studied could be a valuable method for reducing pest population infestations in IPM programmes while being compatible with biocontrol agents.

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Proceedings of the

5th International Symposium on Biological Control of Arthropods (ISBCA-2017)

Edited by Peter G. Mason, David R. Gillespie and Charles Vincent

The 5th International Symposium on Biological Control of Arthropods, held in Langkawi, Malaysia, continues the series of international symposia on the biological control of arthropods organized every four years. The first meeting was in Honolulu, Hawaii, USA in January 2002, followed by the Davos, Switzerland meeting in September 2005, the Christchurch, New Zealand meeting in February 2009, and the Pucón, Chile meeting in March 2013. The goal of these symposia is to create a forum where biological control researchers and practitioners can meet and exchange information, to promote discussions of up-to-date issues affecting biological control, particularly pertaining to the use of parasitoids and predators as biological control agents. This includes all approaches to biological control: conservation, augmentation, and importation of natural enemy species for the control of arthropod targets, as well as other transversal issues related to its implementation.

Topics covered include:

- non-target impacts in biological control as the cornerstone of successful integrated pest management programmes;
- regulation and risk assessment methodology;
- implementing access and benefit sharing policies;
- assessing the impact of biological control programmes for both cost–benefit analyses and determining the socio-economic impact and effect on livelihoods;
- understanding the uptake of biological control solutions in low and lower middle income countries to replace the use of highly hazardous pesticides;
- the role of native and exotic natural enemies; and
- the importance of pre- and post-genetics in biological control.

Cover image: *Apechtis compunctor* (L.) attacking a pupa of *Cydalima perspectalis* (Walker). Photo by Tim Haye.