IAEA-D4.10.25-CR-3
LIMITED DISTRIBUTION

WORKING MATERIA L

Dormancy management to enable mass-rearing and increase efficacy of sterile insects and natural enemies.

Report of the Third Research Coordination Meeting of an FAO/IAEA Coordinated Research Project, held in Vienna, Austria, from 29 May to 2 June 2017

Reproduced by the IAEA
Vienna, Austria 2017

NOTE

TABLE OF CONTENTS

BACKGROUND

Insect pests cause significant and widespread damage worldwide, and insecticides remain the predominant method of control. According to FAO, 30-40 % of agricultural production is lost to pre-harvest and post-harvest infestations, mainly caused by insect pests. Despite growing worldwide dependence on agrochemicals, suppression of pest populations is frequently inadequate. In addition, due to regulation, pest resistance, and environmental and human health concerns, there is an increasing demand for the replacement of the intensive use of these chemicals by environmentally friendly, cost effective and sustainable methods within integrated pest management approaches. Chief among these are biological control applications based on the use of sterile insects and natural enemies. Enhancing the efficacy of biological control requires an understanding of life-history phenology of the target pest or beneficial species.

Most insects face times of the year when reproduction or development are suppressed due to a lack of resources or unfavourable environmental conditions. Dormancy responses have evolved to mitigate the stresses of these unfavourable times and to synchronize insect life cycles with favourable periods. Dormancy responses can include both pre-programmed, hormonally mediated diapause, and also quiescence induced directly by the environment (e.g., low temperatures, drought, lack of hosts, etc.). Quiescence is a state of developmental arrest that can occur in any life stage. In contrast, diapause is a stage-specific developmental arrest that can be either facultative (determined by token stimuli) or obligate (occurring regardless of prevailing environmental conditions).

Many univoltine pest species have an obligatory diapause that synchronizes them with resource availability. For such univoltine insect pest species, sterile insect technique (SIT) and augmentative natural enemy control have not been either practical or possible due to obligatory diapause responses that prevent or interfere with continuous mass rearing. Examples include the European cherry fruit fly, apple maggot fly, Chinese citrus fruit fly, Russian melon fly, and processionary moths. Although obligatory diapause has been a major roadblock to developing biological control programs for many pests, current research suggests that there are approaches that can potentially disrupt obligatory diapause and facilitate mass rearing. Four approaches that appear particularly promising for circumventing the challenges of obligate diapause include: 1) simple environmental manipulations, such as thermal shock, 2) chemical or hormonal treatments, such as application of organic solvents, 3) choosing geographical populations without diapause or artificial selection for non-diapausing strains within populations, and 4) genetic modification by mutagenesis or transgenesis of critical genes for diapause. Successfully circumventing obligate diapause with any of these approaches, or a combination thereof, would provide new opportunities for effective mass rearing of important pest species.

Beyond dormancy there is a spectrum of responses that may increase the stress tolerance of insects, and many of these responses could possibly be exploited for mass rearing and biological control. For example, rapid cold-hardening responses can be induced in active insects. Similarly, variations in the storage environment (for example, via fluctuating thermal regimes) can significantly improve low temperature tolerance. Although insects stored at low temperatures are likely in a quiescent or dormant state, there remain opportunities to increase the duration and reduce the impacts of low temperature exposure via manipulations of storage conditions or physiological states.

While obligate diapause is an obstacle in some cases, two aspects of dormancy and other physiological tolerance responses can be effectively exploited to improve the efficacy of biological control programs.

- First, dormancy can be used to stockpile mass-reared insects and to time the supply of biological control agents to coincide with seasonal demand for release. The ability to synchronize the supply of control agents with demand is critical for the growing biological control industry. Furthermore, an enhanced understanding of dormancy responses could improve phenological models for both pest species and beneficials. Inducing dormant states or other physiological-conditioning treatments opens up new opportunities for either enhancing classical cryopreservation of embryos (in liquid nitrogen) or developing new methods for long-term cold storage of other life stages, such as larvae or pupae. Development of such techniques from an organismal or biochemical perspective could make it feasible to maintain strains over the long term without compromising the genetic integrity of those strains, while avoiding the efforts and costs involved in continuous rearing. This ability to maintain stocks without continuous rearing is especially important when considering the rapid accumulation of mutant and transgenic strains in entomological research laboratories.
- Second, increased stress tolerance is often a hallmark of dormancy, a feature that could be exploited in biological control applications. The efficacy of biological control, including sterile insect programs and natural enemy releases, is affected by the quality of insects released into the field. Poor performance of insects used in field releases can be a product of stresses experienced at multiple points during the production, marking, irradiation, shipping, and release process. The ability to specifically induce dormant states, including either diapause or quiescence, could potentially reduce the above stresses, thereby improving the performance of individuals in field releases. For example, some diapausing insects are known to be resistant to low-level irradiation. Perhaps diapause could be exploited to reduce off-target irradiation damage, outside of germ-line genomic DNA, and improve the performance of sterile insects. Similarly, insects are frequently exposed to mechanical disturbance, hypoxia, and thermal stress during shipping, stresses that may be mitigated by inducing dormant states prior to shipping.

Beyond applications to existing biological control tactics, there is an opportunity to develop the knowledge base needed to generate novel strategies for controlling pest populations by managing diapause and dormancy responses. For example, new approaches to prevent diapause, terminate diapause, or prolong diapause could be exploited to desynchronize insects from favorable environmental conditions, thus inducing "ecological suicide" in pest populations.

Dormancy management and biological control

Developing dormancy management tools could be important for biological control involving sterile insects or natural enemy releases. Eight key questions should be addressed:

1. Can dormancy, physiological conditioning, or storage conditions be used to manage insect life cycles to enable or improve the efficacy of mass rearing?
2. Can dormancy, physiological conditioning, or storage conditions be used to maintain the genetic integrity of laboratory strains?
3. Can an understanding of how environmental variation influences dormancy and physiological tolerances enhance our ability to build phenology models that forecast life-history transitions in pest and beneficial populations?

4. Can dormancy responses, physiological conditioning, or storage conditions be used to enable or enhance the shelf life of beneficial insects including: sterile insects, natural enemies, and pollinators while making them available for release upon demand?
5. Can dormancy responses, physiological conditioning, or storage conditions be used to reduce radiation injury and enhance sterile insect performance?
6. Can dormancy responses, physiological conditioning, or storage conditions be used to decrease shipping-related damage and enhance post-shipping performance of beneficial insects including biological control agents and pollinators?
7. How do microbes and insects interact to determine thermal performance and dormancy of SIT, IIT, pest, and beneficial insects?
8. Can a greater understanding of dormancy responses or physiological conditioning foster the development of novel approaches for insect pest management, for example "ecological suicide"?

These questions are expanded in the following paragraphs:

Can dormancy responses, physiological conditioning, or storage conditions be used to manage insect life cycles to enable or improve the efficacy of mass rearing?

A critical challenge for mass-rearing efforts is that many pest species undergo an obligate diapause that prevents continuous rearing. Many obligate diapause responses necessitate a long period of cold storage to fulfil the biological requirements for continuation of development. This requirement for prolonged cold exposure introduces substantial time gaps that either present an obstacle for mass rearing or decrease the efficiency of the process. Reducing or circumventing the requirement for prolonged cold storage would substantially enhance the efficacy of mass rearing for such species. Several strategies may be used to manage diapause responses. First, diapause may be prevented by environmental, hormonal, or chemical manipulations. Second, insects may have their dormancy terminated prematurely through environmental, hormonal, or chemical manipulations. Third, genetic manipulations may be used to generate non-diapausing strains. These genetic manipulations include a variety of approaches including: artificial selection of strains, exploitation of natural geographic variation for strain foundation, and mutagenic or transgenic approaches for strain development.

Can dormancy responses, physiological conditioning, or storage conditions be used to maintain the genetic integrity of laboratory strains?

The loss of genetic variation and inadvertent artificial selection are challenges for all insect mass-rearing. Long-term rearing over many generations often leads to animals adapted to the mass-rearing environment, but leaving them less able to perform in the field. Dormancy responses may be used to preserve the original genetic integrity of strains, including heterozygosity and rare allelic diversity, as well as the specific genetic architecture of selected strains. Both diapause and other forms of physiological conditioning may be used to exploit new strategies for cryopreservation at very low temperatures in liquid nitrogen (-196^{o}C). Although most cryopreservation has focused on embryos, new strategies focusing on other life stages, such as larvae or pupae, may be effective. Another complementary strategy may be low-temperature cold storage (+10^{o}C to -10^{o}C) of either diapausing or non-diapausing embryos, larvae, pupae, or adults to maintain genetic integrity and to reduce the high costs of strain maintenance by continuous rearing.

Can an understanding of how environmental variation influences dormancy and physiological tolerance enhance our ability to build phenology models that forecast life-history transitions in pest and beneficial populations?

A fundamental aspect of biocontrol programmes is that the release of control agents within a field setting is synchronised with the appropriate developmental stage of the target species, e.g. adult (reproductive) stage for SIT, or host developmental stage for control with parasitoids. This is also relevant to synchronise pollinator release with flowering time. Being able to predict seasonal life history transitions under different environmental scenarios is therefore extremely valuable and will be greatly facilitated by the development of phenology models. The programming of diapause represents a pivotal point in the timing of life history events, and our investigations of how environmental variability influences the decision to enter diapause, diapause duration, the transition to post-diapause quiescence, and subsequent emergence will provide key parameters for these models.

Can dormancy responses, physiological conditioning, or storage conditions be used to enable or enhance the shelf life of sterile insects and natural enemies while making them available for release upon demand?

For both sterile insects and natural enemies, efficient deployment of these insects is achieved when their release coincides precisely with the appearance of pest populations in the field. Unfortunately, there is often a timing gap between the production of sterile insects or natural enemies and the demand for these agents in field releases. The ability to stockpile insects in a dormant state without loss of performance and to mobilize them quickly upon demand is essential for a viable biological control industry using sterile insects or natural enemies.

Can dormancy responses, physiological conditioning, or storage conditions be used to reduce radiation injury and enhance sterile insect performance?

While irradiation effectively induces the dominant lethal mutations necessary for germ-line sterility, irradiation also produces off-target somatic cellular damage that can decrease performance of sterile males in the field. The efficacy of sterile insects would be enhanced substantially if off-target effects of irradiation could be reduced. Increased stress resistance is a characteristic of dormant states and physiological conditioning in insects, thus applying radiation in the dormant state or after physiological conditioning may protect against off-target effects generated during irradiation.

Can dormancy responses, physiological conditioning, or storage conditions be managed to decrease shipping-related damage and enhance post-shipping performance of biological control agents?

Long-distance shipping from mass-rearing facilities to release sites is a common element encountered in the deployment of biological control agents. Shipping can be stressful because mass-reared insects are exposed to multiple stresses including hypoxia, altered barometric pressure, mechanical disturbance, and extremes of high and low temperature. The enhanced stress resistance displayed by dormant insects and insects after physiological conditioning may mitigate some of these perturbations and prevent shipping-related declines in performance of both sterile insects and natural enemies.

How do microbes and insects interact to determine thermal performance and dormancy of SIT, IIT, pest, and beneficial insects?

Insects carry a diverse array of microbes, both eukaryotic and prokaryotic, including symbionts, commensals, and pathogens. These microbes can occur externally, in the gut, and internally, including those microbes that are housed intracellularly. This microbiota can enhance nutritional efficiency, modify physiological responses to a range of stresses, alter interactions with pathogens, and influence reproductive compatibility. The composition and impact of the microbiome can be temperature-dependent. In addition, immune responses constitute part of the generalised stress response, can be activated by a range of physiological stressors, and are likely essential for survival in the field, particularly in high-diversity tropical environments. Beyond the general stress response, there may be components of immunity that are specifically triggered by dormancy, physiological conditioning, or low-temperature storage. Composition of the microbiome in mass-reared insects is an area of growing research interest, as is the manipulation of intracellular microbes in insect control (Incompatible Insect Technique, IIT). Yet we know little about how the microbiota affects thermal biology and dormancy, and vice versa. Thus, understanding the role played by the microbiome in dormancy, physiological conditioning, and cold storage may open new avenues to improve mass-rearing techniques via microbiome manipulation, but this approach has not been explored.

Can a greater understanding of dormancy responses or physiological conditioning foster the development of novel approaches for insect pest management, for example "ecological suicide"?

Insect development is finely tuned to exploit favorable seasons of the year. Altering developmental patterns at the population level by preventing dormancy, breaking dormancy prematurely, or by delaying dormancy termination disrupts synchronization of the insect with its environment, making it vulnerable to unfavorable environmental conditions. This critical seasonal synchronization of dormancy with environmental conditions may be disrupted using hormonal, chemical, or genetic techniques. This knowledge could serve as a base for developing new strategies for pest insect population control.

Can a greater understanding of dormancy responses be used to refine and harmonize methods to assess risk associated with arthropod release?

Thermal responses and dormancy are also major factors determining the potential of a released biological control agent to establish in a new area. Whereas an introduced natural enemy needs to establish for a classical biological control program to be successful, in augmentative biological control establishment is not desired. Particularly after the case of the invasive harlequin ladybird *Harmonia axyridis*, there is increasing concern that non-indigenous species released in augmentative biological control programs may cause undesired environmental effects should they establish and spread. Assessing dormancy and thermal responses is therefore crucial to predict the success of a classical biological control release, whereas it is an essential step in the risk assessment of a non-indigenous augmentative biological control agent.

CO-ORDINATED RESEARCH PROJECT (CRP)

This Coordinated Research Project (CRP) is based on a Consultants Meeting that was held from 7-11 May 2012 in Vienna, Austria (report available) to assess the potential for conducting co-ordinated R&D in dormancy management and cold tolerance of insects with the potential use in SIT application, and to formulate a proposal for a CRP on *Dormancy Management to Enable Mass-rearing and Increase of Sterile Insects and Natural Enemies*.

The overall objective of this new **CRP D4.10.25**, approved for the **period 2014-2019**, is to understand and harness dormancy management, physiological conditioning, and cold-storage approaches to enable mass-rearing of insect species previously difficult to rear and to enhance current mass-rearing efforts for biological control, specifically using sterile insects and natural enemies as part of an environmentally friendly, area-wide integrated pest management approach.

THIRD RESEARCH CO-ORDINATION MEETING (RCM)

Twenty-two scientists from 12 countries attended this third RCM, held in 29 May to 2 June 2017, Vienna, Austria. The list of participants, which included CRP contract and agreement holders, as well as 5 additional observers, is given in **Annex 1**. The agenda for the meeting is attached in **Annex 2**.

During the the meeting, general discussions were held to define and review the thematic areas of the CRP, the review of the general and specific R&D objectives to be addressed during the 5 years of the CRP (**Sections 1 and 2 of this document**), and the revision of the CRP Logical Framework. Furthermore, participants were divided into two working groups to develop more detailed R&D plans to be conducted during the next 18 months of the CRP.

1. MANIPULATING DORMANCY RESPONSES

1.1. Background Situation Analysis

When considering dormancy responses there is both diapause and environmentaly induced quiescence. The seasonal biology of dormancy in any particular species may include both diapause and quiescent periods. Manipulating seasonal biology to facilitate mass rearing will require understanding the three phases of diapause, induction, maintainance and termination, plus any related quiescence responses - all of which can be influenced by temperature (Figure 1). Our long-term goals are to:

- Eliminate the obligate diapause of target species to facilitate mass-rearing programs to support the development of new biological-control programs.
- Induce diapause to stockpile biological control agents, and then terminate diapause on demand to synchronize availability of agents with need in the field.
- Provide insights that would improve modelling the field phenology of pests and beneficial organisms, including improving risk assessment for released arthropods.

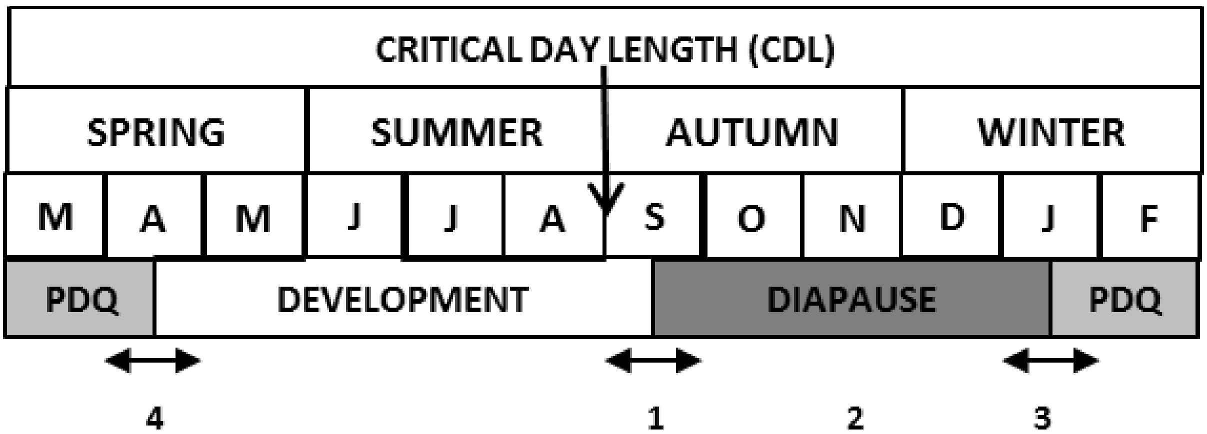

Figure 1. Schematic representation of a 'typical' seasonal phenology (life cycle transition) for a species with facultative winter diapause induced by critical day length (CDL) in late summer in the Northern Hemisphere. Stages of development/activity, diapause and post-diapause quiescence (PDQ) are shown. Arrows indicate that the timing of transition points between these stages can shift in either direction depending on temperature. While overwintering diapause is typically induced by CDL, this response can be masked by warm temperatures in several species. Developmental rate between induction and diapause initiation (1) is also influenced by temperature. This means the seasonal timing of diapause initiation can be strongly temperature dependent. The relationship between temperature and diapause development/the duration of diapause maintenance (2) is complex (Bale and Hayward, 2010), but in virtually all species, the thermal conditions experienced during diapause will influence the timing of diapause termination (3). Once in PDQ, the organism is able to resume development (4) as soon as temperatures cross the appropriate developmental threshold. Thus, the resumption of development and timing of the subsequent adult stage is also temperature dependent. We propose to determine the effects of temperature on the timing of events 1-4 in a range of species within controlled lab environments. This will allow us to determine key thermal thresholds that influence life history transition points and so develop much more accurate phenology models than are currently available. The purpose of these models will be to better predict field population responses to different environmental scenarios and so enhance the efficacy of biocontrol, SIT and pollinator release.

Achieving these outcomes will require substantial advances in our knowledge of the regulation of dormancy responses and exploitation of that knowledge to artificially manipulate dormancy. We will address these long-term goals through research on four sub-themes.

1. Genetics of dormancy responses
2. Environmental treatments that may affect dormancy responses
3. Pharmacological manipulations that may affect dormancy responses
4. Performance outcomes of dormancy management

1.1.1. Genetics of dormancy responses.

Current Knowledge

Decades of research has shown that many dormancy responses have an underlying genetic basis, dormancy responses tend to be polygenic, there is substantial genetic variation within and among populations, and this variation is maintained by local adaptation due to spatial and temporal variation. We lack a detailed understanding of the genetic architecture of many dormancy responses and specifically diapause. This understanding of dormancy genetic architecture could facilitate the development of improved strains with respect to dormancy characteristics by selective breeding or genetic modification.

Gaps identified:

- Dormancy responses are typically polygenic, but multigenic models of diapause have not been well developed particularly with respect to pleiotropic and epistatic genetic interactions.
- Few studies have applied modern genetic tools to characterizing the genetic architecture of dormancy, leaving us with poor resolution of genetic architecture that hinders our ability to identify mechanisms of diapause regulation, or to selectively breed strains with diapause characteristics of interest.
- Interactions between genetic, epigenetic, and environmental factors require substantially more study to predict the life-history stage and environmental conditions in which dormancy may more effectively be manipulated.

1.1.2. Environmental treatments may affect dormancy responses

Current Knowledge

Environmental cues play critical roles in the induction, maintenance and termination of facultative diapause responses. Although less obvious, obligate diapausing species may also respond to environmental variation. For example, holding temperature prior to diapause or during diapause may affect the proportion of individuals that terminate diapause early versus late. Environmental inputs including temperature, photoperiod, host and enemy cues, and physical stimulation can affect diapause initiation and termination. Novel environmental manipulations may be a particularly useful route to manipulate dormancy. Obligate diapause can be eliminated by either preventing diapause induction or by driving early termination.

Gaps identified

- The mechanisms triggering induction in both facultative and obligate diapausing species are poorly known, but represent promising targets for manipulation.

- The underlying mechanisms of diapause termination in both facultative and obligate species remain poorly characterized, but represent promising targets for manipulation.
- To understand how environmental factors are translated through physiological machinery to govern dormancy responses we must explore: modes of action of environmental manipulations, periods of sensitivity within the lifecycle, the effective magnitude and duration of manipulations, and whether manipulations that are used at the research scale are deliverable to larger scales, such as in a mass-rearing facility.

1.1.3. Pharmacological manipulations of dormancy responses.

Current Knowledge

Pharmacological manipulations offer a promising set of tools for facilitating mass rearing of obligate-diapausing species and for regulating facultative dormancy to enhance shelf life of biological control agents and other beneficial insects, like pollinators. Although some pharmacological manipulations have been very successful in either averting diapause completely or terminating diapause, for example applications of ecdysteroids, juvenoids, or hexane, several gaps in our knowledge are currently preventing wide-scale use of pharmacological manipulation of diapause across species.

Gaps identified

- The mechanisms triggering induction in both facultative and obligate diapausing species are poorly known, but represent promising targets for pharmacological manipulation.
- The underlying mechanisms of diapause termination in both facultative and obligate species remain poorly characterized, but represent promising targets for activation to facilitate mass rearing of obligate-diapausing species, and to promote synchronization of biological control agents and beneficials with demand using pharmacological manipulations.
- To understand how pharmacological agents affect the physiological machinery to affect dormancy responses we must explore: modes of action, periods of sensitivity within the lifecycle, the effective doses and durations of manipulations, and whether pharmacological agents that are used at the research scale are deliverable to larger scales, such as in a mass-rearing facility.

1.1.4. Performance outcomes of dormancy management

Current Knowledge

Dormancy responses are often beneficial in promoting survival of organisms through stressful seasons and for synchronizing organismal lifecycles with favourable seasons, like times of resource availability. However, there can be substantial costs of undergoing dormancy. Across many systems with facultative dormancy, individuals that have undergone dormancy show reductions in important life-history parameters compared to non-diapause individuals when held in the same, benign conditions including: survival, energetic reserves, fecundity, fertility, flight, etc. Furthermore, there can be strong ecological costs of averting diapause when needed, or disrupting phenologies by terminating diapause too early or too late. Biological control agents must retain adequate performance after any environmental or pharmacological manipulations to remain effective. Thus, the performance impacts of any manipulation of dormancy must be carefully parameterized.

Gaps identified

- We know little about the effects of averting diapause in obligate-diapausing species on subsequent performance and timing of other life-history events like sexual maturation.
- We know little about the effects of novel environmental or pharmacological manipulations that induce or terminate diapause on subsequent performance and life-history traits.
- We know little about how multiple stressful portions of an integrated biological control program will interact with dormancy manipulation. For example, will dormancy management techniques improve or degrade the response of an individual to other stressors in a biological control program, such as the stresses of shipping and field release, or irradiation in the context of SIT.
- We know little about how altering the genetic architecture of dormancy responses by selecting for non-diapause or facultatively diapausing lines may affect other life-history and performance traits, in addition to inbreeding effects.
- We see an opportunity to use knowledge about dormancy resposnes to improve and harmonize risk assessment protocols for released arthropods, including sterile insects, natural enemies, and other beneficial arthropods.

1.1.5. Critical knowledge gaps across projects

Across projects we will take several approaches for filling critical knowledge gaps, including:

- Selection of non-diapause strains. Even obligate-diapausing species will often have some small portion of the population that fails to enter diapause and instead continues development, or portions of the population that will terminate diapause early relative to the rest of the population. Selective breeding experiments using these non-diapausing or short-diapausing individuals may be particularly useful for addressing both genetic architecture and physiological mechanisms of diapause regulation. Production of non-diapause or short-diapausing lines could facilitate our understanding of the genetic architecture of diapause through breeding designs to estimate heritability and segregation ratios, and QTL mapping to identify candidate genome regions. Furthermore, these lines and the progeny of crosses between non-diapause and diapausing lines may facilitate dissecting the physiological architecture of diapause responses. Selection of non-diapause lines may also provide a near-term benefit by providing lines to be used in mass-rearing programs.
- Exploiting naturally segregating variation in diapause induction or termination timing. Variation in diapause has been demonstrated to segregate among individuals both within and among populations in numerous species of arthropods. Characterizing this variation phenotypically and through associations with population-genetic markers will provide insights into the genetic architecture of diapause and additional opportunities for artificial selection of non-diapause or short-diapause strains.
- Applying environmental manipulations. Changes in the ambient environment, including exposures to high heat and long photoperiods, among others, are well known to affect whether insects enter diapause and the duration of diapause (Figure 1). Dose-response curves for the magnitude and duration of environmental manipulations affecting diapause induction or duration will be estimated for several species. Dose-response experiments should carefully consider the ecological relevance of parameters used and the applicability of manipulations to eventual mass rearing. Beyond traditional single-

factor, single-exposure manipulations, some treatments should explore synergistic effects of multiple exposures and exposure to multiple environmental factors, as well as environmental varibility.

- Applying pharmacological manipulations. Pharmacologial manipulations that either trigger endogenous physiological pathways, such as endocrine signals for diapause, or cause suifficient stress may dissuade the induction of diapause or promote the early termination of diapause. Critical periods for compound application, dose-response curves, and estimations of effects of pharmacological agents on performance of treated insects must be considered carefully. Investigators should bear in mind that there may be synergistic effects between different pharmacological treatments or between pharmacological treatments and environmental manipulations for diapause regulation.

1.2. Individual plans

Table 1. Sub-thematic areas of manipulating dormancy responses being addressed by researchers and the respective insect pest and natural enemies species to be studied (for main areas of work, names appear in bold).

Sub-Theme	Researchers	Insect Species
Genetics of dormancy responses	**Greg Ragland**	*Rhagoletis pomonella*
	Christian Stauffer	*Ips typographus*
	Nikos Papadopoulos	*Rhagoletis cerasi*
	P. De Clercq/T. Van Leeuwen	*Tetranychus urticae* (mites)
Responses to environmental treatments	Dan Hahn	*Rhagoletis pomonella*
	Dave Denlinger	*Sarcophaga bullata*
	Changying Niu	*Bactrocera minax*
	Vlad Kostal	*Chymomyza costata*
	Bin Chen	*Delia antiqua*
	P. De Clercq/T. Van Leeuwen	*Tetranychus urticae & Polyphagotarsonemus latus* (mites) & *Tuta absoluta*
	Shin Goto	*Riptortus pedestris & Dianemobius nigrofasciatus & Nasonia vitripennis*
	George Yocum/ J. Rinehart	*Megachile rotundata*
	Scott Hayward	*Calliphora vicina*
	Juan Rull	*Rhagoletis* spp.
	Nikos Papadopoulos	*Rhagoletis cerasi*
	Nikos Kouloussis	*Eurytoma* spp.
	David Dolezel	*Pyrrhocoris apterus*
Responses to pharmacological manipulation	**Dan Hahn**	*Rhagoletis pomonella*
	Nikos Papadopoulos	*Rhagoletis cerasi*
	Changying Niu	*Bactrocera minax*
	Dave Denlinger	*Culex pipiens*
	Scott Hayward	*Calliphora vicina*
Performance outcomes of dormancy management	George Yocum/ J. Rinehart	*Megachile rotundata*
	Scott Hayward	*Calliphora vicina*

1.2.1. Genetics of dormancy responses

Participants (from the *I. typographus* diapause group): Martin Schebeck, Christian Stauffer, Gregory Ragland, Edwina Dowle, Axel Schopf

5-year plan

- Assessing the genetic basis of facultative and obligate diapause in the spruce bark beetle *Ips typographus* and inferring evolutionary conclusions
- Collection of three European populations
- Defining diapause phenotypes under controlled laboratory conditions
- Preparation of ddRADSeq libraries and sequencing
- Analysis of population genetic structure, especially with regard to diapause strategies, and phylogeographic conclusions
- Using knowledge on diapause strategies of European *Ips typographus* to estimate the influence of this pest species on forests ecosystems

Next 18-months plan

- Performing final bioinformatic analyses, especially a Genome Wide Association Study, to associate a diapause phenotype with a genotype
- Estimating the genetic population structure with regard to the diapause phenotype
- Estimating the composition of diapause phenotypes in the studied populations
- Assessment of the ancestral diapause strategy
- Planning of future work plan, i.e., screening of additional populations, how the acquired information can be included in management strategies for practical forestry

Achievements by the 3rd RCM

- Collection of beetles from European populations
- Defining diapause phenotypes under controlled laboratory conditions
- ddRAD library preparation
- Sequencing of ~ 600 samples
- Initial data analyses

Participants: Greg Ragland, Dan Hahn

5-year plan

- Genetic basis of the propensity of the apple maggot *Rhagoletis pomonella* to enter into diapause and dormancy termination timing with respect to its contribution to phenological differences
- Contribution of diapause-associated loci to host-race differenctiation
- Test for pleiotropy in loci associated with diapause induction versus termination and determine whether the associations across the phases of the diapause developmental trajectory are independent
- Integration of genomic analyses with transcriptomic and endocrine analyses

18-month plan

- Continued analysis of genetic associations for diapause initiation and termination timing using dd- Rad markers for additional diapause phenotypes

Achievements by the 3rd RCM:

- Collection of sympatric field populations of *Rhagoletis pomonella* from both apple and hawthorn fruits
- Library preparation and sequenceing of dd-Rad markers
- Data analyses and interpretation led to first published manuscript in this series

---

Participants: Nikos Papadopoulos, Greg Ragland, Dan Hahn

5-year plan

- Identidy mechanisms associated with diapause termination in *R. cerasi* pupae
- Identify genetic associations with prolonged dormancy in *R. cerasi*

18-month plan

- Collect populations from well-described field sites in Greece and Germany
- Dd-Rad-marker genotypes will be associated with emergence timing across the populations
- Complete RNAseq analyses.

Achievements by the 3rd RCM:

- Samples for RNA extraction and transcriptional characterization using RNA-seq during diapause termination manipulations have been collected.
- RNAseq libraries have been constructed, sequenced, assembled, and the data preprocessed prior to in-depth analyses.
- Emerging adults have been collected for genomic DNA extraction
- Informatics analyses are in progress

Participants: Astrid Bryon, Thomas Van Leeuwen

5-year plan

- Exploration of g-protein coupled receptors in diapause regulation
- Exploring the extent to which host-plant cues affect spider mite diapause

18-month plan

- Inducing diapause in albino mites via diets, backcrossing with wildtype or other thermal regimes
- Unravel the function of the high concentration of keto-carotenoids during diapause: carrying out comparison experiments with albino mutant line (fecundity, UV protection, oxidative stress, cold tolerance, etc.)

Achievements by the 3rd RCM:

- Expression of putative spider mite antifreeze proteins in a yeast system, followed by functional testing – attempted and expressed AFP but did not observe antifreeze activity
- Verifying the influence of photoperiod and temperature on diapause responses in western European strains of *P. latus* and *T. absoluta*
- Identified a carotenoid-synthesis enzyme laterally transferred from a microbe that is associated with diapause induction in spider mites and pigmentation in other mites

1.2.2. Environmental treatments

Participants: Dan Hahn, Greg Ragland

5-year plan

- Compare transcriptional patterns between the apple and hawthorn host races of *Rhagoletis pomonella* at the point in pupal diapause development when the two host races diverge in their response to diapause-terminating cues

18-month plan

- Complete data analyses from RNA-seq experiments

Achievements by the 3rd RCM:

- Collection of sympatric field populations of *Rhagoletis pomonella* from both apple and hawthorn fruits
- Identified timepoints duing the diapause developmental program when host-races diverge in their responses to termination cues in preparation for RNA-seq experiments.
- Harvested brain tissue and ring glands from >200 pupae of each host race.
- Produced RNAseq libraries, sequenced these libraries, and performed assembly and preprocessing of these data.

Participant: David Denlinger

5-year plan

- Complete microRNA analysis to identify candidate microRNA's for diapause programming, diapause termination, and maternal effects on diapause induction in the flesh fly *Sarcophaga bullata*
- Complete functional studies on the gluconeogenic control enzyme PEPCK to interrogate its possible role as a regulator of intermediary metabolism during diapause

18-month plan

- Begin functional studies of microRNAs, using computational methods, microRNA mimics and sponges, as well as identification of potential targets of microRNAs.
- Complete analysis of PEPCK transcripts and functional analysis

Achievements by the 3rd RCM:

- MicroRNA sequencing completed and results analysed, showing potential targets relevant to the diapause phenotype.
- Identified two microRNAs that are upregulated during diapause and thus are expected to function in downregulating their gene targets.
- Identified eight microRNAs that are downregulated during diapause, suggesting they may function to upregulate their target genes.
- Additional microRNAs appear to be specifically expressed in association with a diapause maternal effect.

- Roles of acetylation and deacetylation evaluated biochemically and by monitored expression of key genes involved in a form of histone modification that appears to be related to diapause.
- Two variants of PEPCK characterized; description of the tissue and developmental distributions of the two variants completed; results show dynamic progression of expression during diapause.

Participant: Changying Niu

5 year plan

- To investigate the molecular mechanisms underlying pupal diapause induction and termination in *Bactrocera minax*.
- Determine whether stress tolerance is enhanced by diapause programming.

18-month plan

- assess the gene expression of the main components in insulin signaling pathway in distinct tissues during diapause development
- evaluate the biological function of neuropeptide in the regulation of diapause transition
- measure the main nutrient contents across diapause stages and analyze their fluctuations with regarding to diapause transition

Progress completed by the 3rd RCM

- RNAi experiments for several genes were performed and will be continued to explore new genes in pupal diapause termination in *B. minax*.
- Transcriptional patterns for pupal diapause termination in *B. minax*.

Participant: Patrick De Clercq

5-year plan

- Investigate the cold tolerance of invasive pests in Europe (emphasis on *D. suzukii*)
- Investigate cold tolerance in key insect and mite predators intended for biological control releases in Western Europe, in search of cold tolerant strains.
- Assess cold tolerance as part of the establishment potential of exotic arthropod biological control agents or exotic insects used for food/feed in the framework of risk assessment
- Investigate the role of rearing methods (diets) on cold tolerance of beneficial arthropods.

18-month plan

- Evaluate lethal times (0°C) + CCRT for adults of *D. suzukii* as a function of larval diet (artificial diet vs. fruits from wild/cultivated hosts) and investigate food*morph interaction
- Field cage experiments on cold tolerance of *D. suzukii*
- Starvation and dessication tolerance of *D. suzukii* when fed on fruit

---

Participant: Vlad Kostal

5-year plan

- Use RNA-seq to identify candidate transcripts for photoperiodic induction of larval diapause in the fly *Chymomyza costata*.

18-month plan

- Monitor the expression of clock gene transcripts and localization of clock proteins in the brains of *C. costata* in the context of photoperiodic diapause induction.

Achievements by the 3rd RCM:

- We characterized the transcription profiles associated with photoperiodic diapause induction in the larvae of the drosophilid fly *Chymomyza costata* and we identified candidate genes and processes linked to upstream regulatory events that eventually lead to a complex phenotypic switch from direct ontogeny to larval diapause.

Participant: Bin Chen

5-year plan

- Analysis of transcripts and pathways associated with summer and winter diapause in *Delia antiqua* using RNA-seq
- Comparison of the possible molecular mechanisms between winter and summer diapauses
- Genome-wide identification and expression verification of candidate genes in diapause-associated pathways
- Functional study of candidate genes in diapause-associated pathways by RNAi

18-month plan

- Meta-analysis of gene expression patterns in diapause initiation, maintenance and termination of winter and diapauses.
- Comparison of the possible molecular mechanisms between winter and summer diapauses
- Identification of immune genes of onion fly, *Delia antiqua*, and gene expression pattern analyses under the non- and summer-diapausing states
- Regulation of Transcription Factor FOXO1 on Lipolysis gene *Brummer* in Summer Diapausing Pupa of *Delia antiqua*

Achievements by the 3rd RCM:

- Collected onion maggot samples to study diapause initiation, maintenance and termination of winter and diapauses
- Performed RNA sequencing of the above samples to compare gene expression patterns in diapause initiation, maintenance and termination of winter and diapauses
- Described trehalose metabolism in the diapausing stage of *Delia antiqua*, including gene cloning, characterization, and expression and enzymatic activities related to trehalose metabolism
- Completed and published work on RNAseq analyses of summer diapause in *Delia antiqua*

- Completed and published a follow-up study to our RNAseq analysis of sumer diapause that implicated cuticle genes as being important to describe cuticle protein gene family expression under non-diapause and winter-diapasue conditions
- Completed and published a follow-up study to our RNAseq analysis of sumer diapause that implicated HSP genes as being important to describe HSP23 transcript abundance patterns in non-diapause, as well as both winter and summer diapausing *Delia antiqua*

Participant: Shin Goto

5-year plan:

- Clarify physiological and molecular regulation of dormancy in the bean bug, *Riptortus pedestris*.
- Verify roles of the circadian clock genes on various photoperiodic responses, including lipid accumulation, body coloration, and cold tolerance.
- Identify circadian clock cells and neural pathways governing photoperiodism
  - Identify candidate genes for photoperiodism by RNAseq
  - Test the function of candidate genes with RNAi.
- Comparative studies to investigate the roles of the circadian clock genes in dormancy in the cricket *Dianemobius nigrofasciatus* and the parasitoid wasp *Nasonia vitripennis*.

Next 18-month plan:

- Identify clock-regulated, JH-independent cascades regulating photoperiodic regulation of diapause in the bean bug.
- Identify neural pathways integrating photic information in the brain in photoperiodism in the bean bug.
- Identify circadian clock cells governing photoperiodic time measurement in the jewel wasp.

Achievements by the 3rd RCM:

- Clarify clock-regulated, JH-independent lipid accumulation in the bean bug.
- Clarify clock oscillation pattern under thermoperiodic conditions promoting promoting eclosion phase changes in the onion fly.
- Clarify the significance of the clock gene *period* in photoperiodic induction of larval diapause in the jewel wasp.
- Clarify involvement of pars intercerebralis neurons in oviposition in the bean bug.
- Identify the brain region containing pigment-dispersing factor-immunoreactive neurons and its significance in the photoperiodic induction of reproductive diapause in the bean bug.

Participants: Joe Rinehart, George Yocum, Kendra Greenlee

5-year plan

- Interrogate the transcriptional landscape of diapausing prepupae of the alfalfa leafcutting bee, *Megachile rotundata*, under both laboratory and field conditions

18-month plan

- Use RNA-seq to characterize the differences between early and late season bees

- Investigate the use of low temperature FTR (with a base below zero) to extend diapause/post-diapause quiescence

Achievements by the 3rd RCM:

- We used RNA-seq to characterize candidate transcripts for identifying molecular signatures of separate phases of diapause development.
- We used RNA-seq to determine transcriptional differences between lab- and field-stored *M. rotundata*, which show effects of the timing of diapause initiation and the environment of diapause maintenance.

Participant: Scott Hayward

5-year plan

- Molecular profiling of the diapause programme under different environmental manipulations to identify putative regulatory mechanisms of dormancy and enhanced stress tolerance in the fly *Calliphora vicina* and the parasitoid wasp *Nasonia vitripennis*
- Use understanding of how environmental variability influences diapause to develop phenological models of life history transitions under different climate scenarios
- Analysis of population differences in diapause responses (metabolomics and RNAseq) across latitudinal gradients to further characterise regulatory mechanisms that might underpin the decision to enter diapause

18-month plan

- Undertake analysis of metabolomics data from *N. vitripennis* time series
- Publish synthesis of *C. vicina* phenology model
- Acquire funding for population comparison study

Achievements by the 3rd RCM:

- Completed collection of metabolomics data from time series analysis of trans-generational signals underpinning diapause vs. non-diapause developmental pathways in *Nasonia vitripennis*
- Metabolomic analysis of *C. vicina* data is largely complete
- Phenology model for *C. vicina* has been completed as P. Coleman's thesis

---

Participant: Juan Rull, Rodrigo Lasa

5-year plan

- Characterize adult emergence patterns for different populations (host-races) and species in the genus *Rhagoletis* and associated parasitoids (including the distribution of emergence times).
- Explore whether there is facultative diapause, quiescence, or adult longevity during host scarcity periods for *Solanum* infesting multivoltine *Rhagoletis*.
- Characterize adult emergence patterns across several thermal treatments to better predict emergence for laboratory studies.

- Explore the effect of different environmental cues (temperature, humidity, photoperiod, host-plant derived chemicals) on survival and duration of dormancy.

18-month plan

- Complete data recovery for the experiment on the effect of photoperiod on early and late hawthorn infesting populations of Mexican *R.pomonella*.
- Examine the effect of artificial winter length on survival and duration of dormancy of Mexican populations of *R. completa* and associated parasitoids.
- Examine the effect of host plant cues (e.g. juglanone) on adult eclosion and duration of dormancy for species of walnut infesting *Rhagoletis* (*R. completa* or *R. zoqui*).
- Examine the effect of pre-winter length on survival and adult eclosion of Mexican populations of *R. pomonella*.

Achievements by the 3rd RCM:

- The effect of artificial winter length on survival and duration of dormancy was documented for Mexican populations of *R. pomonella*, *R. zoqui*, *R. turpiniae*, and *R. solanophaga*.
- The effect of winter length on survival, duration of dormancy, and propensity to engage in prolonged dormancy was documented for two Mexican populations of *R. cingulata,* and *R. turpiniae*.
- Pupae of two Mexican *R.pomonella* populations were exposed to different day lengths in an attempt to increase the number of non-dormant individuals and document the effect of photoperiod on survival and duration of dormancy
- Second attempt to select non dormant *R.pomonella* to establish a strain for SIT purposes

---

Participant: Nikos Papadopoulos

5-year plan

- Address patterns of post-dormancy development in different *R. cerasi* populations
- Test for effects of temperature cycles and thermal shocks (short exposure to very cold or hot temperatures) on diapause termination and prolonged dormancy of *R. cerasi* pupae
- Explore the phenomenon of prolonged dormancy and test several low temperatures for suitability for long-term storage of *R. cerasi* pupae
- Develop a management protocol that, employing *R. cerasi* biotypes expressing different diapause intensity levels and seasonal emergence timing, could be used to produce a near continuous supply of adult flies for SIT applications over a period of several months that can cover the demands of most European countries

18-month plan

- Collect populations from well-described field sites in Greece and Germany to test the effect of the length of high temperature pre-winter regimes on first year dormancy length and induction of prolonged 2-year dormancy (see 5-year plan)
- Develop a management protocol that, employing *R. cerasi* biotypes expressing different diapause intensity levels and seasonal emergence timing, could be used to produce a near continuous supply of adult flies for SIT applications over a period of several months that can cover the demands of most European countries

- Explore effect of short chilling shocks and/or thermocycles on diapause termination of at least one *R. cerasi* population

Achievements by the 3rd RCM:

- Showed that extended prewinter intervals affect diapause termination and prolonged dormancy induction
- Showed that prewinter temperature regime affect diapause termination and prolonged dormancy
- Showed that chilling and pre-chilling and chilling intervals affected diapause termination and prolonged diapause schedules in one Greek population of *R. cerasi*
- Effects of the diapause intensity, population and dormancy type on the post dormancy development of *R. cerasi* pupae have been determined

---

Participant: Nikos Kouloussis

5-year plan

- Manipulation of diapause development for improving control of *Eurytoma plotnikovi* wasp (pistachio seed wasp)
- Study the prolonged diapause in *Eurytoma plotnikovi*

18-month plan

- Determine the timing of diapause termination in field conditions - ongoing
- Determine the timing of diapause termination in lab conditions - ongoing
- Estimate the size of larvae by weight and determine whether there are any associations coaitions with diapause phenotype
- Assess morphology, weight and longevity of adults that diapause for a second year
- Investigate the effect of photoperiod and cold storage in larvae that have a prlonged diapause
- Investigate the effect of photoperiod on diapause development during cold storage in individuals with prolonged diapause

Achievements by the 3rd RCM

- Showed that extended periods of chilling affected post-winter performance and survival
- Determined diapause termination period under field and lab conditions
- Measured morphology, weight and longevity in adults that diapause for one year
- Showed that long day photoperiod favours diapause termination
- Showed that long day photoperiod favors diapause development during cold storage

---

1.2.3. Pharmacological manipulation

Participants: Dan Hahn, Greg Ragland

5-year plan

- Quantify juvenoid and ecdysteroid titers in diapausing pupae of both apple and hawthorn host races of *Rhagoletis pomonella*.
- Test the extent to which treatments of juvenoids and ecdysteroids can be used to artificially terminate diapause and assess whether the two host races that differ in their refractoriness to terminate diapause also have different effective doses for exogenous hormonal stimulation of termination.

18-month plan

- Collection of sympatric field populations of *R. pomonella* with both apple and hawthorn fruits.
- Continue to develop our methods to quantify juvenoids by HPLC-MS

Achievements by the 3rd RCM:

- We can now reliably quantify femptomolar quantities of ecdysone and 20-hydroxyecdysone based on our work with the apple host-race flies. Juvenoids have been more difficult to quantify, but we will continue to develop these techniques.
- We have already completed substantial field collections in the fall of 2016 where we were able to collect sufficient numbers of apple and hawthorn flies to perform our desired comparisons.
- We have collected all of the data on ecdysone and 20-hydroxy ecdysone contents of prewinter, overwinter, and post-winter pupae of both host races and we are currently analysing these data to determine whether additional sampling of both host races in necessary in the fall of 2017.

Participant: Nikos Papadopoulos

5-year plan

- Test the effects of hormonal and/or other chemical treatments on diapause regulation of *R. cerasi* pupae.

18-month plans

- Test the effects of hormonal and/or other chemical treatments on diapause regulation of *R. cerasi* pupae.

Participant: Changying Niu

5-year plan

- To investigate the molecular mechanisms underlying pupal diapause induction and termination in response to pharmacological agents in *Bacrtocera minax*.
- Verify expression patterns of candidate genes induced by 20-HE treatment via qRT-PCR.

- Use RNAi to manipulate expression patterns of candidate genes induced by 20-HE treatment.
- Screen known inhibitors of the signaling tranduction pathway for activity in maintaining or terminating diapause
- To investigate the diapause regulation of parasitic wasps in *Bacrtocera minax* in response to 20E treatment.

18-month plan
- determine the 20E concentration of diapausing individuals after different cold chilling for different durations.
- analyze the non-genomic actions of 20E regarding to diapause transition
- identify the membrane receptors by searching against previously constructed transcriptome data and assess their functions in response to 20E treatment by RNAi
- verify the signal transduction pathways involved in diapause transition by using various inhibitors

Progress completed by the 3rd RCM
- Differentially expressed proteins in response to 20E treatment at different time points have been identified
- Integrated analyses with omics data have been performed and several pathways were selected as candidates involving in diapause transition.
- Samples from cold chilling experiments for quantification of 20E titres were collected and will be finished in the following months.
- Use 20-HE treatment to terminate diapause and produce adults in the laboratory for use in development of an artificial diet for mass rearing.
- Understand the responses of diapausing individuals in different diapause statuses to 20HE application, as well as 20HE-response gene expression verification.

Participant: <u>David Dolezel</u>
3-year plan
Genetic basis of photoperiodic timers and role of circadian genes in diapause regulation in *Pyrrhocoris apterus*:
- Identify circadian gene homologs in *P. apterus* and test if these genes are functioning in the circadian clock. Test what circadian genes are involved in the photoperiodic timing and which are not.
- Identify neuropeptide toolkit of *P. apterus* and test what neuropeptides are involved in *P. apterus* reproduction and reproductive diapause.
- Identify, whether *P. apterus* photoperiodic clock measures day-length or night-length

18-month plan
- Test function of selected circadian genes in the circadian clock and in the photoperiodic timing by RNAi and/or gene editing
- Test role of temperature on insects' activity and specifically explore temperature compensation of circadian clock and temperature-modulated daily activity
- Test whether *P. apterus* photoperiodic clock measures day-length or night-length

Progress completed by the 3rd RCM

- Explored role geographic diversity of circadian clocks in *P. apterus* by analyzing free running period of 59 field-lines originating from various geographical localities Test role of temperature on insects' activity and specifically explore temperature compensation of circadian clock and temperature-modulated daily activity
- functionally tested role of circadian genes in nymphal induction of diapause

---

Participant: David Denlinger

5-year plan

- Decipher additional links in the pathway leading from photoreception to the endocrine events dictating the diapause response in the mosquito *Culex pipiens*

18-month plan

- Characterize additional genes that are potentially regulated by the transcription factor FOXO

Achievements by the 3rd RCM:

- Over 70 genes potentially involved in diapause regulation appear to bind to FOXO, leading to the diapause phenotype
- Demonstration that the circadian clock and a downstream gene *pigment dispersing factor* are essential for diapause manifestation
- Described an effect of the imidazole derivative in enhancing the incidence of diapause and increasing diapause duration in several insect species

---

Participant: Scott Hayward

5-year plan

- Select metabolic targets for direct pharmacological manipulation (diet or injection) and assess efficacy in establishing diapause-like phenotypes in non-diapause insects and/or to disrupt diapause

18-month plan

- Ongoing dietary supplementations and assessment of stress tolerance or disrupted diapause phenotypes
- Publish metabolomics study

Achievements by the 3rd RCM:

- Undertaken dietary manipulations using selected metabolites from metabolomics screen, including QC of dose responses

---

1.2.4. Performance outcomes of dormancy management

Participant: **Joe Rinehart, George Yocum, Kendra Greenlee**

5-year plan

- To develop treatments that maximize post-diapause performance in the commercial pollinator *Megachile rotundata*

18-month plan

- Asses reproductive ability in the field after storage treatments
- Determine the mechanism of hypoxia-induced improvements in long-term storage

Achievements by the 3rd RCM

- We assessed flight ability after diapause/quiescence extension by FTR.
- We determined the thermoperiods that best synchronize adult emergence.

Participant: Scott Hayward

5-year plan

- Assess post-diapause (adult) performance consequences of environmental and pharmacological manipulations in the fly *Calliphora vicina and Osmia bicornis*.

18-month plan

- Ongoing assessment of adult performance (stress tolerance, activity thresholds, fecundity etc.) following environmental and pharmacological manipulations.
- Expand assessments of adult performance in *O. bicornis* to address stakeholder requirements (e.g. assessments of pollinator performance).

Achievements by the 3rd RCM:

- Completed initial assessments of adult phenotypes in *C. vicina* following dietary supplementation of larvae.
- Completed initial assessment of adult performance (longevity, survival) in *O. bicornis* in response to pharmacological/environmental manipulations.

---

2. LOW TEMPERATURE BIOLOGY

2.1. Background and situation analysis

Low temperature storage or treatment is an integral part of the rearing or release protocols in many biological control programs. In addition, although insects may be reared under optimal thermal conditions, they may be released into the field under thermal conditions that may differ substantially from the conditions under which they were reared. Therefore, an understanding of insect thermal biology has the potential to enhance the performance of insects reared for release. We identify four main areas in which thermal biology can contribute to rearing and storage.

1. Treatment conditions and manipulations that improve insect cold tolerance.
2. Facilitation and enhancement of survival and performance under long-term cold storage, including cryopreservation.
3. Enhancement of the thermal performance of insects destined for release.
4. Manipulation of thermal biology to cause 'ecological suicide' in a pest control context.

We recognise that there may be a gap between the development of potential methods and their practical implementation. The purpose of the CRP is to identify key parameters that could enhance storage and release; the translation of these findings to practical use will be a problem for the future.

2.1.1. Treatment conditions and manipulations that improve insect cold tolerance

<u>Current knowledge</u>

Insects use a few main strategies to tolerate low temperatures: most insects can not survive body freezing and use mechanisms to prevent their bodies from freezing when they are exposed to sub-zero temperatures (freeze intolerant) while a number of other species can tolerate being frozen (freeze tolerant). Insect thermal tolerance is highly plastic, and responds to a range of external cues during development, after exposure within a single life stage, and after brief exposures within a short period of time (Table 2).

Cold injury has a range of causes, including disruption of ion balance, injury to cell membranes, metabolic perturbations, and mechanisms underlying plasticity include variation in the production of cryoprotectants, antifreeze proteins, membrane composition, alterations in a number of metabolic pathways, and behavioural modifications that ultimately affect cold tolerance (Figure 2). In addition, cold tolerance can be transferred between generations, and from host to parasitoid, although the mechanisms underlying this information transfer are unknown. Cold tolerance can also be improved by more artificial interventions, such as hyperprolinemia via dietary loading.

Table 2: List of endogenous and exogenous factors that may affect phenotypic plasticity in cold tolerance. After Colinet and Boivin (2011, *Biological Control* 58, 83-95).

Exogenous Factors	Endogenous Factors
Temperature	Mass and body reserves
Duration of exposure	Life-history strategy
Rating of cooling or heating	Nutrition
Gradual acclimation	Mode of reproduction
Rapid acclimation	Age/stage
Acclimatization	Dormancy status
Developmental temperature	Sex
Constant and fluctuating cold exposure	Physiological history
Combined cold exposure	Reproductive status
Humidity	
Photoperiod	
Chemicals	
Modified atmospheres	
Handling & rearing stress	
Crowding	
Microbial pathogens & symbionts	
Inherited environmental effects	

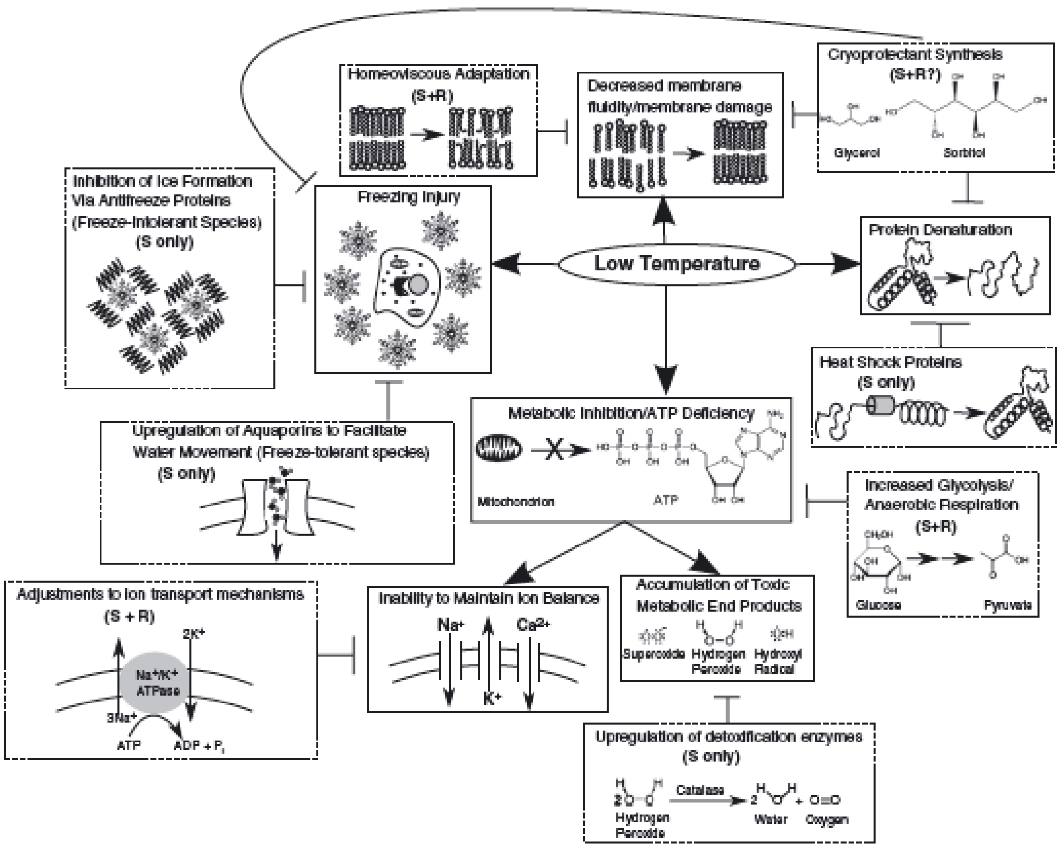

Figure 2: Mechanisms of low temperature damage and repair in insects. From Teets and Denlinger (2013 *Physiological Entomology* 38, 105-116).

During a rearing-and-release protocol, insects may be exposed to sub-optimal temperatures at several points: at collection, during radiation exposure, during packing for transport, during transport, and as part of the release.

Gaps Identified

- What are the mechanisms underlying cold injury, recovery and repair of injury, and plasticity of thermal tolerance?
  - Understanding the mechanisms of cold injury may allow us to prevent damage, to enhance the speed of recovery from cold exposure, to specifically induce responses that improve thermal tolerance, or to identify novel approaches for enhancing performance after cold exposure during the protocol.
- Can physiological conditioning be used to improve survival and performance?
  - Physiological conditioning can be used to increase cold tolerance of insects prior to exposure to cold. This can include thermal treatments, photoperiod changes, and exposure to other stresses that may induce cross tolerance or hormesis.
  - In addition, diet manipulation in preparation for cold exposure has the potential to shorten recovery time, improve survival, or enhance performance
- Can cold tolerance be transferred among generations or between trophic levels?
  - It is increasingly apparent that the environmental history of parental generations can affect the thermal tolerance of their offspring, and that thermal tolerance can be passed from host to parasitoids. However the prevalence and mechanisms of these relationships are poorly understood.
- What is the role of epigenetic processes in determining thermal tolerance?
  - Epigenetic mechanisms, involving small RNAs, DNA acetylation, DNA methylation, and histone modification can drastically alter patterns of gene expression, transduce information between life stages and generations, and alter responses to environmental cues. There is evidence for epigenetic regulation in diapause regulation, and while cold-specific microRNAs have been identified for goldenrod gall flies, epigenetic regulation has not been well-explored in other systems.
- What is the role of the microbiome and immunity in cold hardiness?
  - There is a wealth of recent literature that suggests that the microbiome has significant impacts on the biology of their host animals, across a wide range of taxa. In insects, we know that endosymbiotic bacteria (and even some pathogens) can enhance thermal tolerance. Although there is some evidence that insect immune systems respond to cold, the role of the microbiota in cold tolerance has not been explored.
- How do dormancy processes interact with cold tolerance?
  - Although dormancy and quiescence are closely associated with the development of cold tolerance in many species, understanding how much the genetic and physiological architecture are shared between traits could allow diapause or cold hardiness to be independently modified.

2.1.2. Facilitate and enhance survival and performance under cold storage.

Current Knowledge

During cold exposures of days to weeks (or even months to years), insects can accumulate indirect chilling injuries. In some insects, these injuries can be prevented by pre-treatments that include exposure to mild cold. In addition, it is now well-established that fluctuations in the thermal environment, for example, periodic bursts of exposure to permissive temperatures (Fluctuating thermal regimes, FTR), can enhance cold survival and even abate the accumulation of injury. The mechanisms by which FTRs reduce cold injury are not well-known, but are subject to ongoing investigation. In addition, many insects are capable of surviving long exposures to very low temperatures, and there is the potential to use understanding of those mechanisms to optimise long-term storage (for example, to stockpile reared insects for future release), or to identify novel molecules and processes to enhance existing cryopreservation protocols (used to maintain genetic diversity in populations reared for release).

Gaps Identified

- What are the mechanisms by which FTRs improve survival?
    - Understanding these mechanisms will allow us to develop treatments that use FTRs to enhance survival during long-term cold storage and improve post-storage performance.
    - Understanding the mechanisms underlying chilling injuries may be particularly useful for identifying treatments that ameliorate long-term chilling damage.

- Can FTRs be optimized to improve cold storage of mass-reared insects?
    - Currently most FTRs utilise a daily pulse at permissive temperatures. Practical concerns about warming and cooling make less frequent interruptions of cold exposure attractive. What are the minimum and maximum durations and intervals for FTRs that can elicit enhanced chilling tolerance?
    - The fitness and metabolic costs of the FTR responses have not been well-explored, and the consequences for field performance must be considered, as must the potential for FTRs to elicit deacclimation or development during the FTR regime.

- Can fluctuations in environmental parameters other than cold during storage enhance survival?
    - Currently, most fluctuating regimes that affect cold tolerance primarily use daily exposures to permissive temperatures. The potential for fluctuations in photoperiod, humidity, and other environmental factors to improve chilling tolerance has not been well-explored.
    - Other physical, diet, or chemical manipulations, for example dietary amino acid loading or hypoxia, could also act in a manner similar to FTRs to abate chilling injury.

- Can reliable markers for insect quality be identified?
    - There exists a general need for biomarkers to determine the effects of cold storage on the quality of insects destined for release. The development and implementation of such markers requires not only the identification of the relationships between cold-induced damage and fitness-related parameters such as flight and reproductive performance, but also the development and dissemination of standard protocols.

- o

- Can cryopreservation techniques be enhanced and extended to more species?
  - Cryopreservation procedures have been very successful for a small number of species. There is a need to expand the range of taxa for which cryopreservation is available, and to increase the throughput of existing cryopreservation protocols.
  - Exploration of the mechanisms underlying insect cold tolerance might reveal novel mechanisms and molecules that could be used to enhance or modify existing cryopreservation protocols, or extend cryopreservation to additional life stages or taxa.
- Is IIT compatible with cold storage?
  - *Wolbachia* load can be affected by thermal stress, although this has been well-explored at high temperatures, the impact of low temperatures on intracellular microbes designed to reduce reproductive compatability is unknown. Similarly, the impact of *Wolbachia* infection on cold tolerance is unknown.

2.1.3 Enhancing the thermal performance of insects destined for release.

<u>Current Knowledge</u>
Insects may often be released into environments that contrast significantly with those under which they were reared and stored. It is well-known that there are tradeoffs between upper and lower thermal tolerances (mediated, for example, by modifications to cellular membrane fluidity). In addition, there is a considerable empirical and theoretical understanding of the evolutionary and ecological implications of acclimation, although the mechanisms underlying cold acclimation in insects are less well-understood.

<u>Gaps Identified</u>

- Can we identify mechanisms underlying thermal acclimation and deacclimation?
  - Understanding these mechanisms may allow high-low temperature tradeoffs to be ameliorated, or for laboratory selection to allow decoupling of high temperature tolerance (necessary for field survival) from survival of low temperature storage.

- Can we optimize the timing of release to enhance performance?
  - Thermoperiod, photoperiod, atmospheric pressure, and endogenous circadian rhythms all affect insect performance. There exists capacity to use these rhythms to optimise the performance of the insects upon release.
  - Enhancement of thermal performance could also be season-specific, for example thermal conditioning could be used to enhance low temperature performance of early-season releases, where insects will encounter lower temperatures than at mid-summer.

- Can we develop treatments that increase field competitiveness?
  - A key component of all thermal manipulations is that they can affect performance in the field. There exists a need to extend measurement of post-cold performance beyond survival to include field performance (see also measures of quality, above).

2.1.4 Manipulating thermal biology to cause ‘ecological suicide’ in a pest control context.

Current Knowledge and identified gaps

We have a reasonable knowledge of the scope and range of plasticity of insect cold tolerance, as well as the capacity to use this knowledge to enhance thermal tolerance (see sections 1-3). This knowledge could also be harnessed to disrupt thermal tolerance in field populations of pest insects. This approach has been explored for ice-nucleating bacteria to reduce overwinter cold tolerance (albeit with limited success), but has not been widely explored in other systems.

2.2. Individual plans

Table 3. Sub-thematic areas of low temperature biology being addressed by researchers and the respective insect pest and natural enemies species to be studied (for main areas of work, names appear in bold).

Sub-Theme	Researchers	Insect Species
Treatment conditions and manipulations that improve insect cold tolerance	Dave Denlinger	*Nasonia vitripennis & Sarcophaga crassipalpis*
	Changying Niu	*Bactrocera dorsalis*
	Hervé Colinet	*Drosophila melanogaster*.
	Brent Sinclair	*Gryllus pennsylvanicus & Gryllus veletis & Drosophila* spp.
	Mariana Viscarret	*Trichogramma nerudai* & *Trichogramma pretiosum*
	J. Sørensen/J.Overgaard	*Trichogramma* spp. & *Drosophila subobscura*
	Scott Hawyward	*Calliphora vicina*
	Patrick De Clercq	*Tuta absoluta & Epitrix* spp. & *Polyphagotarsonemus latus & Cryptolaemus montrouzieri*
	Mahbab Hasan	*Plodia interpunctella*
Facilitate and enhance survival and performance under long-term cold storage	Mariana Viscarret	*Trichogramma nerudai* & *Trichogramma pretiosum*
	Vlad Kostal	*Drosophila* spp.
	Hervé Colinet	*Drosophila melanogaster* & *Alphitobius diaperinus*
	George Yocum/J. Rinehart	*Megachile rotundata*
	Brent Sinclair	*Gryllus* spp. & *Eurosta solidaginis*
Enhancing the thermal performance of insects destined for release	Martin Wohlfarter	*Cydia pomonella*
	Brent Sinclair	*Gryllus veletis*
	Mariana Viscarret	*Trichogramma nerudai*
	J. Sørensen/J.Overgaard	*Trichogramma* spp.
	Scott Hayward	*Calliphora vicina* & *Bombus terrestris*
	George Yocum/J. Rinehart	*Megachile rotundata*
	Eunhye Ham, Junseok Lee	*Monochamus saltuarius*
	John Terblanche	*Thaumatotibia leucotreta*
Manipulating thermal biology to cause 'ecological suicide' in a pest control context	**Dave Denlinger**	*Heleothis* spp. & *Helicoverpa* spp.

2.2.1. Treatment conditions and manipulations that improve insect cold tolerance

Participants: Hervé Colinet, Rui Cardoso Pereira, Kostas Bourtzis and Carlos Cáceres
5-year plan

- Extend FTR application to species of interest for SIT and/or IIT and test whether cold storage (under constant temperature or FTR) is compatible with irradiated and Wolbachia-infected insects.
- Assessing how acclimation treatments and/or nutritional variables can be optimally exploited to prepare *Drosophila* spp., including these of interest for SIT and/or IIT, for short term cold storage.

24-month plan

- Identify acclimation treatment/duration that is enough/necessary to reach high level of cold tolerance and identify physiological correlates associated with the action of acclimation using RNAseq, lipidomics and untargeted metabolomics.

Achievements by the 3rd RCM:

- Characterise interactions between FTRs and acclimation in *D. melanogaster* in preparation for cold storage.
- Assessment of basal thermal tolerance of European populations of *D. suzukii* and optimizing storage conditions under constant and/or fluctuating cold conditions

---

Participant: Patrick De Clercq

5-year plan

- Investigate the cold tolerance of invasive pests in Europe (emphasis on *D. suzukii*)
- Investigate cold tolerance in key insect and mite predators intended for biological control releases in Western Europe, in search of cold tolerant strains.
- Assess cold tolerance as part of the establishment potential of exotic arthropod biological control agents or exotic insects used for food/feed in the framework of risk assessment
- Investigate the role of rearing methods (diets) on cold tolerance of beneficial arthropods.

24-month plan

- Evaluate lethal times (0°C) + CCRT for adults of *D. suzukii* as a function of larval diet (artificial diet vs. fruits from wild/cultivated hosts) and investigate food*morph interaction
- Field cage experiments on cold tolerance of *D. suzukii*
- Starvation and dessication tolerance of *D. suzukii* when fed on fruit

---

Participants: David Denlinger, Vlad Koštál
5-year plan

- Metabolomic studies to determine the physiological effects of dietary manipulation on host and parasitoid to identify potential future dietary manipulations.

24-month plan

- no new activity in this area planned.

Achievements by the 3rd RCM:

- Addition of proline to the diet of the flesh fly enhanced cold tolerance in the parasitoid *Nasonia vitripennis*.
- Metabolomics studies described major shifts in fly metabolism following envenomation by *Nasonia* and the supplemention of diets with proline.

Participant: Mahbab Hasan

5-year plan

- Determine the cold tolerance of *T.evanescens* and *Bracon hebetor* within host eggs and mature larvae of Indian meal moth (IMM) respectively under specific environmental and developmental parameters. Assess whether chilling has latent effects on the quality of the adult parasitoid
- Determine the most effective method for cold storage of unparasitized IMM eggs by examining post-storage acceptability by the parasitoid, parasite survival, reproduction, and host-seeking behavior
- Evaluate the efficacy of extending the shelf-life of unparasitized eggs and mature of IMM by cold storage.
- Establish the diapause induction, maintenance and termination in host (IMM) and parasitoids (*B. hebetor* and *T. evaescens*)
- Assess the performance of parasitoids *B. hebetor* developing from diapause and non-diapause IMM mature larvae
- Evaluate effects of prolonged cold exposure of juvenile IMM on adult performance.

Plan for next 18 months

- Establish the methods for photo phase based diapause induction, maintenance and termination in IMM final instar larvae
- Develop a method to establish diapause induction, maintenance and termination in parasitoids (*B. hebetor* and *T. evanescens*)
- Assess the performance of parasitoids (*B. hebetor* and *T. evanescens*) developing from the diapause
- Determine lethal time of IMM eggs and mature larvae at 0 degC
- Determine relationship between exposure duration at 0 degC and chill coma recovery time in IMM mature larvae
- Determine the effects of short (4 h) and long (12h) pre-exposure to 0 degC on CCRT and survival at 0 degC of IMM eggs and mature larvae
- Determine whether the pre-exposure treatments protect performance of IMM adults that were cold-exposed as eggs and/or larvae.

Achievements by the 3rd RCM:

- Established a suitable technique for the mass rearing of host and parasitoid.
- Developed methods for cold storing of IMM eggs prior to enhancing the production of quality parasitoid.
- Established the methods for diapause induction, maintenance and termination in IMM final instar larvae.
- Assessed the performance of parasitoids developing from diapause and non-diapause IMM mature larvae.

- Three graduate students have completed their MS thesis relating to the research aspects of diapause and biological control of IMM.

Participant: Scott Hayward

5-year plan

- Identify –omic signatures associated with improved stress tolerance
- Directly manipulate target molecules and pathways to modify cold tolerance of *Calliphora vicina* (as a model system) to identify targets in other species.

24-month plan

- Ongoing analysis of metabolomics data and identification of metabolites potentially contributing to low temperature tolerance. Expand dietary supplementation to other species (*O. bicornis*)

Achievements by the 3rd RCM:

- Completed collection of samples from metabolomics screen to identify candidates underpinning enhanced cold tolerance.

Participant: Vladimír Koštál

5-year plan

- Extend acclimation treatments to additional species of interest for SIT and to beneficial insects.

24-month plan

- Characterise the effectiveness of FTRs for inducing acclimation in *Drosophila* in preparation for cold storage.
- Evaluate dietary manipulation and acclimation to improve cold tolerance of Drosophilid flies

Achievements by the 3rd RCM:

- We optimized the cold acclimation and cooling protocol which significantly improved freeze tolerance in the larvae of *D. melanogaster*. We identified an interval of low temperatures under which the larvae can be stored in quiescence for up to two months. We have found that compared to larvae stored at constant low temperatures, the larvae stored under FTR conditions were able to decrease the rates of depletion of energy substrates, and more efficiently exerted protection against oxidative damage and accumulation of indirect chill injuries.

Participant: Changying Niu

5-year plan

- Diets manipulation by pharmacological agents or cold acclimation tests to boost cold adaptation ability in the case of *Chrysomya megacephala* and *Bactrocera dorsalis*.
- Measure the effects of cold acclimation on survival, longevity, fecundity of *C. megacephala*.
- Investigate the cold tolerance and phenotypic plasticity in the case of *C. megacephala* for application in greenhouse agriculture and early spring season. In addition, the cold acclimation tests of *B. dorsalis* will better explain its fast disperse towards cold areas.

24-month plan

- Determine the supercooling points and cold tolerance strategy; estimate the lower lethal temperature for *C. megacephala* and *B. dorsalis*.
- Measure the critical thermal minimum and investigate chill coma recovery for *C. megacephala* and *B. dorsalis* after cold-hardening/acclimation.
- Assess cold tolerance of *C. megacephala* and *B. dorsalis* overwintering potential.
- Test the rapid cold hardening/long time acclimation on the cold tolerance in terms of SCPs and survival rate of *C. megacephala* and *B. dorsalis*

Progress by the 3rd RCM

- We obtained some data in *B. dorsalis* related to its cold tolerance but need to repeat.

Participant: Nikos Papadopoulos,

5-year plan

- Explore cold tolerance in different population and developmental stages of *C. capitata*
- Explore effects of *Wolbachia* infection on cold tolerance of *C. capitata*
- Explore the cold tolerance of different developmental stages of *R. cerasi*

24-month plan

Ceratitis capitata

- Define the cold tolerance of adult, pupae and L3 larvae of laboratory and wild populations
- Explore effects of *Wolbachia* infection on cold tolerance of *C. capitata*
- Effect of thermal shocks on demographic and behavioural properties

Rhagoletis cerasi

- Explore the cold tolerance of larvae, pupae and adults
- Explore effects of adult age and adult food on cold tolerance

---

Participant: Jan Rozsypal, Vlad Koštál

5-year plan

Identification and assessment of factors which enable and which limit survival in frozen state in insects (*Pyrrhocoris apterus*, *Cydia pomonella*, *Drosophila melanogaster*, *Chymomyza costata*):

- Examine the mechanism and possible ecological relevance of inoculation by external ice for freezing survival in insects.
- Examine how dynamics of ice formation and amount of ice influence survival in frozen state and find if the amount of ice differ between tissues/body compartments.
- Examine the effect of acclimation and metabolomic composition on survival in frozen state in whole insects and their tissues/cells.

24-month plans

- Assess the ecological relevance of freezing survival after inoculation by external ice in *P. apterus* and *C. pomonella* and identify the part(s) of body where ice penetrates inside during inoculation.
- Measure the dynamics of ice formation and amount of ice which forms at decreasing temperatures in whole body and selected tissues (hemolymph, midgut, hindgut…) in *P. apterus* and *C. pomonella* from both field and laboratory cultures.

Participant: Brent Sinclair

5-year plan

- Identify candidate genes associated with freeze tolerance.
- Manipulate gut yeast flora in *Drosophila* spp. and identify species/combinations that affect thermal tolerance or survival/performance.
- Identify treatments that improve cold tolerance of laboratory-reared Asian Longhorned Beetle (*Anoplophora glabripennis)*

24-month plan

- Conduct metabolomics and transcriptomics assays to identify potential candidate genes and pathways associated with freeze tolerance in *Gryllus veletis*.
- Identify candidate pathways and mechanisms to explain the phenotypic impact of gut *Lachancea* yeast on *Drosophila* cold tolerance.
- Compile metabolome and transcriptome of lab-reared diapausing Asian longhorn beetles.

Progress by 3rd RCM

- Publish paper on gut loading to enhance cold tolerance and chill coma recovery in *Gryllus pennsylvanicus.*
- Publish best practices paper on measuring cold tolerance in insects.
- Identify model species for ongoing freeze tolerance studies.
- Develop methods to manipulate gut yeast flora in *Drosophila* species.

Participants: Jesper Sørensen, Johannes Overgaard

5-year plan

- Investigate phenotypes and associated mechanisms induced by FTRs in *Drosophila*.
- Investigate costs and benefits of cold acclimation in *Drosophila* and agriculturally-important species (e.g. *Orius majusculus, Geolaelaps aculeifer, Trichogramma sp.*).

24-month plan

- Measure phenotypic plasticity of cold tolerance in *Drosophila,* including: 1) evaluating the effects of FTR and cold acclimation in relation to homeostatic capacity (in collaboration with Hervé Colinet); 2) identification and validation of mechanisms induced by FTR and mechanisms leading to evolutionary adaptation to FTR.

Achievements by the 3rd RCM:

- Measure phenotypic plasticity of cold tolerance in *Drosophila*. 1) Effects of short term acclimation treatments at different life stages (Paper submitted).
- Measure phenotypic plasticity of cold tolerance in an agriculturally-important species (*Trichogramma*) (paper published).

Participant: Mariana Viscarret

5-year plan

- Establish a pre-storage physiological conditioning protocol to improve stockpiling capacity for *Trichogramma nerudai* and *Trichogramma pretiosum.*

24-month plan

- To study the maternal effect (using long day conditions) on *T. nerudai*
- To study the maternal effect (using long and short day conditions) on *T. pretiosum*
- To improve the cold tolerance in *T. pretiosum* using FTR

Achievements by the 3nd RCM:

- Identify a tractable method to assay cold tolerance in *Trichogramma nerudai* and *T. pretiosum* to allow screening of potential acclimation treatments.
  Manipulate photoperiod in the maternal generation and temperature in the stored generation in a fully-crossed design to examine the effects of physiological conditioning on cold tolerance of *Trichogramma nerudai*.

2.2.2. Facilitate and enhance survival and performance under long-term cold storage.

Participant: Hervé Colinet

5-year plan

- Develop optimal FTR conditions for a range of pest species (including *Drosophila suzukii*).
- Identify candidates associated with the action of FTR, and examine the role of those candidates using Omics methods in *Drosophila*.

24-month plan

- Assessment of fitness and/or metabolic costs of FTRs

Achievements by the 3rd RCM:

- Omic survey of physiological responses to FTRs in *Drosophila*
- Characterise parameters (duration, frequency, return time, temperature, life stage) that are necessary and sufficient to elicit the FTR response to optimise FTR protocol for *Drosophila* sp.

Participant: Vlad Kostal

5-year plan

- Continue effort to develop cryopreservation methods for *Drosophila* larvae and non-embryonic life stages of other insects.

18-month plan

- Measure -omic changes during FTRs in *Drosophila*.
- Evaluate the effect of a range of diet manipulations on freeze tolerance of *Drosophila* larvae.

Achievements by the 3rd RCM:

- We have found the larvae of *D. melanogaster* stored under FTR conditions were able to exploited brief warm episodes of FTR for homeostatic control of metabolite levels. We tested the effect of feeding the larvae with diets augmented with 31 different amino compounds, administered in different concentrations. We have found that proline- and arginine-augmented diets showed the highest potential to improve freeze survival.

Participant: Brent Sinclair

5-year plan

- Identify mechanisms underlying chilling injury in insects.

24-month plan

- Identify morphological and cellular changes in the *Gryllus* osmoregulatory system that are associated with cold acclimation.
- Identify transcripts associated with acquisition of cold tolerance in diapausing Colorado Potato Beetle (*Leptinotarsa decemlineata*).

Progress by 3rd RCM

- Identified transcriptional changes underlying acclimation of the ionoregulatory tissues of the chill-susceptible cricket *Gryllus pennsylvanicus*.

Participant: Mariana Viscarret

5-year plan

- Evaluate FTRs as a means to improve survival of long term cold storage (in collaboration with Hervé Colinet).

24-month plan

- -To assess the maternal effect on diapause induction inT. pretiosum
- Evaluate the performance of these parasitoids under semi controlled conditions after cold storage protocols applied

---

2.2.3. Enhancing the thermal performance of insects destined for release.

Participant: Scott Hayward

5-year plan

- Expand dietary manipulation to include other candidate molecules identified in *C. vicina* metabolomic screens.
- Expand dietary manipulation-performance studies to other species, such as *Bombus terrestris* and solitary bees.

18-month plan

- Ongoing assessment of adult performance associated with dietary supplementation and following thermal manipulation of diapause (*C. vicina* and *O. bicornis*).

Achievements by the 3rd RCM

- Partially completed assessment of *C. vicina* adult performance following dietary manipulation of larvae and adults. Identified lowered CTmin and chill coma temperatures in samples supplemented with alanine, but supplementation with proline is lethal.

Participant: Brent Sinclair

5-year plan

- Determine whether thermally induced immune changes modify performance, survival, and pathogen resistance of insects in the field.

24-month plan

- Determine whether there are seasonal changes in the immune system and gut microbiota of *G. veletis*.

Progress by 3rd RCM

- Determined the capacity of the *G. veletis* immune system to acclimate to different thermal conditions.

Participants: Jesper Sørensen, Johannes Overgaard

5-year plan

- Extend microcosm and semi-field evaluations to agricultural systems in agricultural important pest management species.
- Evaluate the effects of genetic adaptation and regional variation on semi-field performance of thermally-acclimated insects.

24-month plan

- Evaluate the costs and benefits of pretreatments that enhance cold tolerance on performance in *Orius majusculus*.
- Evaluate thermal and associated behavioural performance of commercial stocks and wild conspecifics of *Orius majusculus*.

Achievements by the 3rd RCM

- Evaluate the impact on pretreatments that enhance cold tolerance on performance in field collected and commercially reared mites (*Geolaelaps aculeifer*) ***(paper submitted).***
- Evaluate the impact of pre-treatments that enhance cold tolerance on performance in semi-field conditions in *Trichogramma* (paper published).

Participant: John Terblanche, Minette Karsten

5-year plan

- Determine impacts of chilling intensity and duration on laboratory and field performance of the false codling moth (FCM) *Thaumatotibia leucotreta* for manipulating field performance.
- Determine effects of chilling on longevity, fecundity, ion homeostasis and low temperature activity thresholds in the lab.

24-month plan

- Determine effects of lab thermal acclimation rearing on field release recapture rates across a range of thermal conditions.
- In a sub-set of individuals, determine molecular correlates (e.g. metabolomics and/or transcriptomics) of high recapture rates in FCM and Medfly from field releases and similar thermal conditions in the lab.
- Develop a flight mill system for measuring dispersal in the lab for FCM.
- Measure dispersal traits in FCM (and/or Medfly) on flight mills (or alternative setup) across a range of thermal conditions.
- Determine plastic responses of dispersal traits and potential fitness trade-offs in FCM.

Achievements by 3rd RCM

- CTmin has been estimated for both male and female FCM adults.
- Impacts of developmental thermal acclimation conditions on adult CTmin, fecundity and longevity have been determined.
- Publication accepted: Boersma *et al.* (*in press*) Agric. Forest Ent.
- Model development is still on-going. ____

2.2.4. Manipulating thermal biology to cause 'ecological suicide' in a pest control context.

Participant: David Denlinger

5-year plan

- Evaluate the potential to use diapause hormone and non-peptide cuticle-penetrating diapause hormone mimics on the *Heleothis/Helicoverpa* complex of agricultural pests.

18-month plan

- Screen additional non-peptide libraries for molecules that can penetrate the cuticle and exert a diapause hormone effect.
- Apply potent diapause hormone analogs and antagonists to plant surface and incorporate into artificial diet to evaluate potential uptake and action of peptides and non-peptide analogs for diapause disruption.
- Screen select diapause disruptors for additional biological activity beyond diapause disruption.

Achievements by the 3rd RCM

- Identified a peptide analog capable of penetrating the cuticle to prevent onset of pupal diapause.
- Identification of the diapause hormone receptor.
- Confronting challenges of delivering DH active analogs via food.
- Discovered a role for capa peptide as a regulator of cold and desiccation responses.
- Discovery of a putative antifreeze protein in the sunn pest, *Eurygaster maura*.

3. LOGICAL FRAMEWORK

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
Overall Objective: The objective of the project is to understand and harness dormancy management, physiological conditioning, and cold-storage approaches to enable mass-rearing of insect species previously difficult to rear and enhancing current mass-rearing and quality assurance efforts for biological control, specifically using sterile insects and natural enemies as part of an environmentally friendly, area-wide integrated pest management approach.	N/A	N/A	Member States will continue to suffer major losses to endemic and introduced insect pests. The demand for area-wide integrated insect pest management approaches, including SIT and augmentative biological control as non-polluting suppression/eradication components, continues to increase, mandating expansion and improvement in cost-effectiveness of these environment-friendly, sustainable approaches. International trade in agricultural commodities will continue to increase and be disrupted by pests requiring expensive post-harvest and quarantine measures.

Specific Objectives:	Verifiable indicators	Means of verification	Important Assumptions
1. Develop and assess new methods to manage dormancy responses, physiological conditioning, and storage conditions to facilitate mass rearing.	Research and development focused on generating new methods to manage dormancy to facilitate mass rearing.	Scientific reports and peer-reviewed publications.	Dormancy responses, physiological conditioning, and storage conditions can be managed in many systems to avert dormancy or shorten the length of the dormancy period.
2. Develop and assess new methods to use dormancy responses, physiological conditioning, and storage conditions to maintain the genetic integrity of laboratory strains and improve performance by explicitly considering genetic architecture	Basic and applied research focused on the use of dormancy responses in cryopreservation, long-term cold storage, and performance of arthropods	Scientific reports and peer-reviewed publications.	Genetic variation in dormancy responses, physiological conditioning, and storage conditions can be used to enhance the capacity for cryopreservation and long-term cold storage, as well as to improve arthropod performance to enhance biological control programs.
3. Develop methods to incorporate dormancy into phenological models to improve the timing of field releases.	Research and development focused on improving phenological models.	Scientific reports and peer-reviewed publications.	Dormancy responses can be incorporated into phenological models to improve the timing of field releases of biological control agents.
4. Develop and assess new methods to use dormancy responses, physiological conditioning, and storage conditions to enable or enhance the shelf life of biological control agents, including sterile insects and natural enemies.	Research and development focused on the use of dormancy responses, physiological conditioning, and storage conditions to enhance shelf life.	Scientific reports and peer-reviewed publications.	Dormancy responses, physiological conditioning, and storage conditions can be used to enable or enhance the ability to stockpile and mobilize biological control agents upon demand, without diminishing performance.

5. Assess whether dormancy responses, physiological conditioning, and storage conditions can be used to reduce radiation injury and enhance sterile insect performance.	Research and development focused on the use of dormancy responses, physiological conditioning, and storage conditions to reduce injury and enhance post-irradiation performance.	Scientific reports and peer-reviewed publications.	Dormancy responses, physiological conditioning, and storage conditions can be used to reduce radiation injury to somatic cells of biological control agents while maintaining the sterility of germ-line cells.
6. Assess whether dormancy responses, physiological conditioning, and storage conditions can be used to decrease shipping-related damage and enhance post-shipping performance.	Research and development focused on the use of dormancy responses, physiological conditioning, and storage conditions to reduce shipping-related injury and enhance post-shipping performance.	Scientific reports and peer-reviewed publications.	Dormancy responses, physiological conditioning, and storage conditions can be used to reduce injury incurred during shipping and improve post-shipping performance.
7. Explore the role of the microbiome on dormancy responses, physiological conditioining, and cold storage to enhance mass rearing and shelf life of biological control agents.	Basic research focused on characterizing associations between the microbiome and dormancy responses, physiological conditioning, and storage conditions	Scientific reports and peer-reviewed publications.	The microbiome contributes to dormancy, physiological conditioning, and cold storage responses and that the microbiome can be manipulated to improve the quality of biological control agents.
8. Explore the potential for dormancy responses to generate novel approaches for inducing "ecological suicide".	Basic research focused on developing new techniques and theoretical perspectives for disrupting dormancy responses to control pest insect populations.	Scientific reports and peer-reviewed publications.	Disruption of natural dormancy responses can be used to control insect pest populations by desynchronizing seasonal pests with their environments.
9. Refining and harmonizng methods to assess risk associated with arthropod release	Disseminate existing methods and assess the degree to which laboratory research predicts field responses	Scientific reports and peer-reviewed publications.	Dormancy and thermal responses are important factors for the potential establishment of non-native species or genotypes

Outcomes:	Verifiable indicators	Means of verification	Important Assumptions
1. Mass rearing of pests enabled or enhanced by exploiting dormancy or physiological conditioning.	Protocols applied to mass rear at least 2 species with dormancy or physiological conditioning.	Mass-rearing reports	There is a need to mass rear insects with fixed periods of dormancy and/or improved physiological tolerances.
2. Genetic integrity of strains maintained through dormancy management or physiological conditioning in cryopreservation and long-term cold storage, and genetic architecture of dormancy responses assessed.	Protocols applied for cryopreservation or long-term cold storage for at least 2 species. Genetic architecture of dormancy responses assessed for at least 2 species.	Colony management reports.	Facility staff struggles to maintain the genetic integrity of strains; dormancy management or improving physiological tolerances may increase strain maintenance capacity and decrease costs.
3. Better prediction of phenologies to boost the efficacy of release programs.	Phenology models that include dormancy responses applied to at least 2 species.	Phenology modelling reports.	The ability to synchronize the release of biological control agents with the phenology of targets itn eh field will improve the efficacy of control programs.
4. Dormancy management and/or physiological conditioning in use to enable or enhance the shelf life of biological control agents, including sterile insects and natural enemies.	Protocols applied to stockpile at least 2 biological control strains and at least 1 pollinator.	Colony production and storage reports.	The ability to stockpile insects and mobilize them on demand is important for the viability of biological control and pollination programs.

5. Dormancy management and physiological conditioning used to reduce radiation injury and enhance sterile insect performance.	Protocols applied for evaluation in at least 2 species of biological control agents.	Evaluation reports under operational conditions.	Enhanced stress resistance is a facet of many dormancies and physiological-conditioning treatments, so treated individuals may experience reduced somatic radiation damage during sterilization, while maintaining germ-line sterility.
6. Dormancy management or physiological conditioning used to reduce shipping-induced injury and enhance the performance of sterile insects and natural enemies.	Protocols applied for evaluation in at least 2 species of biological control agents.	Evaluation reports under operational conditions.	Damage incurred during shipping due to hypoxia, mechanical disturbance, and exposure to temperature extremes limits the efficacy of biological control agents in field releases and inducing dormant states or physiological conditioning may reduce such damage.
7. Microbes demonstrated to impact dormancy and cold responses.	Associations between tolerances and microbial communities evaluated in at least 2 species.	Scientific publications and technical reports.	Microbial manipulation could open up new avenues to enhance the performance of sterile insects and natural enemies.
8. The potential for dormancy responses to generate novel approaches for inducing "ecological suicide" evaluated under laboratory conditions.	New perspectives evaluated under laboratory conditions.	Scientific reports and peer-reviewed publications.	There is a need for novel environmentally friendly tools to control pest populations.

9. Assist risk assessors in applying efficient and realistic metrics for predicting establishment potential for released athropods	Disseminate available documents about thermal biology metrics of importance to risk assessors	Report for risk assessors disseminated	There is a need for risk assessors to effectively interpret information about thermal biology and dormancy

Outputs:	**Verifiable indicators**	**Means of Verification**	**Important Assumptions**
1.The potential to terminate or avert unwanted dormancy responses using thermal, hormonal, and chemical manipulations evaluated.	Thermal, hormonal, and chemical manipulations tested in at least 3 species.	Reports and peer-reviewed publications.	Thermal, hormonal, and chemical manipulations that have been successful in other models will be applicable to species important for biological control.
2.The potential for dormancy management to maintain the genetic integrity of important strains, cryopreservation and long-term cold storage techniques assessed, genetic architecur of dormancy assessed.	Protocols developed and assessed in model species and extended to selected pest species.	Reports and peer-reviewed publications.	Recent successes in cryopreservation and long-term cold storage of model species can be extended to species important for biological control.
3.The potential to enhance biological control and beneficial insect release practices with new phenology models evaluated.	Phenology models developed and assessed in at least 2 species.	Reports and peer-reviewed publications.	Phenological models that incorporate dormancy responses will more effective than existing models
4. The ability to enable or enhance storage and mobilization of biological control agents with dormancy management evaluated	Protocols developed and assessed in at least 3 species.	Reports and peer-reviewed publications.	Dormancy responses can be exploited to increase the shelf life of biological control agents while

			maintaining performance.
5.The potential for dormancy to reduce radiation injury and enhance sterile insect performance determined.	Protocols developed and assessed in at least 2 species.	Reports and peer-reviewed publications.	Stress resistance is often enhanced in the dormant state and this can be exploited to reduce radiation-induced injury while maintaining sterility.
6.The potential for dormancy to reduce shipping-related damage and enhance post-shipping performance evaluated.	Protocols developed and assessed in at least 2 species.	Reports and peer-reviewed publications.	Stress resistance is often enhanced in the dormant state and this can be exploited to reduce shipping-related damage while maintaining performance.
7.The potential of the microbiota to improve dormancy responses, physiological conditioning, or cold-storage evaluated	Associations between the microbiota and insect performance assessed in at least 2 species	Reports and peer-reviewed publications.	Understanding and manipulating associations between insects and their microbiome will enhance dormancy, physiological conditioning, and cold storage.
8.Novel perspectives on the role of dormancy disruption in controlling pest populations explored.	Novel theoretical perspectives explored.	Reports and peer-reviewed publications.	Hormonal and chemical manipulations currently under investigation for dormancy regulation could be translated into novel methods for control.
9. Research needed to assess risk of establishment of released arthropods performed, and documents written specifically for the needs of risk assessors produced	Parameters relevant to risk assessment of released insects assessed	Primary publications and synthesis document	Research outputs will be used by risk assessors when relevant

ACTIVITIES:	Verifiable indicators	Means of Verification	Important Assumptions
1. Hold Consultants Meeting.	Consultants meeting held May 2012.	Consultants Meeting resulted in CRP proposal.	Consultants meeting approved.
2. Announce project amongst established entomologists working on seasonal pests and establish CRP.	CRP announced and research contracts and agreements submitted, evaluated, and forwarded to IAEA committee.	Issued contracts and agreements.	Proposals submitted and approved by IAEA committee
3. Organize first RCM to plan, coordinate and review research activities (3rd quarter 2014).	1st RCM held in July 2014.	Working material printed and distributed for 1st RCM.	Research activities commence. Reports published and distributed following each RCM.
4. Carry out R&D.	Research carried out by contract and agreement holders.	Reports and publications.	Research activities continue, progress satisfactory.
5. Second RCM to analyse data and draft technical protocols as required (early 2016)	2nd RCM held in 2016.	Working material printed and distributed for 2nd RCM; Research published in scientific literature and disseminated to member states and scientific community.	Renewal requests and continued funding of RCM's and CRP.
6. In conjunction with the second RCM meeting 3-4 day hands-on workshop on "Tools and techniques for quantifying insect performance in relation to dormancy, physiological conditioning, and cold storage".	Workshop held in 2016. Harmonized procedures and trainees capable of implementing novel techniques.	Workshop report integrated as an appendix to the report of the Second RCM.	There is need for training; techniques and instructors are available.
7. Conduct research and Development	Research carried out by contract and agreement holders.	Reports and publications.	Renewal requests and continued funding of RCM's and CRP.

8. Review the CRP after its third year.	Mid-CRP review carried out.	Report of mid-CRP review.	Mid-CRP review by Agency committee is positive.
9. Convene third RCM to evaluate and standardize protocols (late 2017).	3rd RCM held in 2017.	Working material printed and distributed for 3rd RCM; Research published in scientific literature and disseminated to member states and scientific community.	Mid-CRP review approved by IAEA committee. Research activities continue, progress satisfactory.
10. Continue R&D.	Research carried out by contract and agreement holders.	Reports and publications.	Renewal requests and continued funding of RCM's and CRP.
11. Hold final RCM to review data and reach consensus (early 2019).	Final RCM held in 2^{nd} quarter 2019.	Final CRP report.	Research and dissemination activities concluded.
12. Evaluate the CRP and submit evaluation report.	CRP evaluation carried out.	CRP evaluation report.	CRP evaluation by Agency committee is positive.

13. Summarize and publish advances of CRP in a series of joint publications.	CRP members submit papers summarizing activities.	Publication in scientific literature.	Manuscripts submitted, edited, peer reviewed and published.

4. PUBLICATIONS

Andersen J.L., T. Manenti, J.G. Sørensen, H.A. MacMillan, V. Loeschcke and J. Overgaard. 2015. How to assess *Drosophila* cold tolerance: chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits. Functional Ecology, 29: 55-65.

Benoit J.B., Q. Zhang, E.C. Jennings, A.J. Rosendale and D.L. Denlinger. 2015. Suppression of net transpiration by multiple mechanisms conserves water resources during pupal diapause in the corn earworm, *Helicoverpa zea*. Physiological Entomology 40:336-342.

Bentley M.T., F.M. Oi, S.A. Gezan and D.A. Hahn. 2015. Tunneling performance increases at lower temperatures for *Solenopsis invicta* (Buren) but not for *Nylandaria fulva* (Mayr). Insects. 6: 686-695.

Boardman L., J.G. Sørensen and John S. Terblanche. 2015. Physiological and molecular mechanisms associated with cross tolerance between hypoxia and low temperature in *Thaumatotibia leucotreta*. Journal of Insect Physiology 82: 75–84.

Boardman L., J.G. Sørensen, V. Kostal, P. Simek and J.S. Terblanche. 2016. Chilling slows anaerobic metabolism to improve anoxia tolerance of insects. Metabolomics, 12: 176.

Boardman L., J.G. Sørensen, V. Kostal, P. Simek and J.S. Terblanche. 2016. Cold tolerance is unaffected by oxygen availability despite changes in anaerobic metabolism. Scientific Reports, 6: 32856.

Boersma N., L. Boardman, M. Gilbert and J.S. Terblanche. (*in press*). Sex-dependent thermal history influences cold tolerance, longevity and fecundity in false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). Agricultural and Forest Entomology.

Bonte J., D. Musolin, D. Conlong and P. De Clercq. 2016. Diapause and winter survival of two *Orius* species from southern Africa. BioControl 61: 519.

Cascone P., S. Carpenito, S. Slotsbo, L. Iodice, J.G. Sørensen, M. Holmstrup and E. Guerrieri. 2015. Improving the efficiency of *Trichogramma achaeae* to control *Tuta absoluta*. Biological Control 60: 761-771.

Clemmensen S.F. and D.A. Hahn. 2015. Dormancy cues alter insect temperature-size relationships. Oecologia. 177: 113-121.

Coello Alvarado L.E., H.A. MacMillan and B.J. Sinclair. 2015. Chill-tolerant *Gryllus* crickets maintain ion balance at low temperatures. Journal of Insect Physiology 77: 15-25.

Coleman P.C., J.S. Bale and S.A.L. Hayward. 2015. Protein feeding affects the rapid cold hardening and thermal activity thresholds of adult *Calliphora vicina* (Diptera: Calliphoridae). PLoS ONE: e0131301.

Colinet H., B.J. Sinclair, P. Vernon and D. Renault. 2015. Insects in Fluctuating Thermal Environments. Annual Review of Entomology 60: 123–40.

Denlinger D.L. and P.A. Armbruster. 2014. Mosquito diapause. Annual Review of Entomology 59: 73-93.

Denlinger D.L. and P.A. Armbruster, 2016. Molecular physiology of mosquito diapause. Advances in Insect Physiology 51: 329-361.

Des Marteaux L.E. and B.J. Sinclair. 2016. Ion and water balance in *Gryllus* crickets during the first twelve hours of cold exposure. Journal of Insect Physiology 89: 19-27.

Des Marteaux L.E., A. H McKinnon, H.Udaka, J. Toxopeus and B.J. Sinclair. 2017. Effects of cold-acclimation on gene expression in Fall field cricket (*Gryllus pennsylvanicus*) ionoregulatory tissues. BMC Genomics 18: 357.

Ferguson, L.V. and B.J. Sinclair. (in press). Insect immunity varies idiosyncratically during overwintering. Journal of Experimental Zoology A.

Ferguson L.V., D.E. Heinrichs and B.J. Sinclair. 2016. Paradoxical acclimation responses in the thermal performance of insect immunity. Oecologia 181: 77-85.

Fu D.Y., F.L. Si, B. Chen, Y.L. Xu and Y.J. Hao. 2016. Transcriptome-guided identification of the cuticular gene family of *Delia antiqua* (Diptera: Anthomyiidae) and expression analysis under non-and winter-diapause conditions. Acta Entomologica Sinica 59: 172-184.

Gerken A.R., O.C. Eller, D.A. Hahn, and T.J. Morgan. 2015. Constraints, independence, and evolution of thermal plasticity; probing the genetic architecture of long and short-term thermal acclimation. Proceedings of the National Academy of Sciences. 112: 4399-4404.

Goto, S.G. 2016. Physiological and molecular mechanisms underlying photoperiodism in the spider mite: comparisons with insects. Journal of Comparative Physiology B 186, 969-984.

Guz N., U. Toprak, A. Dageri, M. Oktay Gurkan and D.L. Denlinger. 2014. Identification of a putative antifreeze protein gene that is highly expressed during preparation for winter in the sunn pest, *Eurygaster maura*. Journal of Insect Physiology 68: 30-35.

Hand S.C., D.L. Denlinger, J.E. Podrabsky and R. Roy. 2016. Mechanisms of animal diapause: recent developments from crustaceans, insects, nematodes and fish. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology 310: R1193-R1211.

Hao Y.J, Y.J. Zhang, F.L. Si, D.Y. Fu, Z.B. He and B. Chen. 2016. Insight into the possible mechanism of the summer diapause of *Delia antiqua* (Diptera: Anthomyiidae) through digital gene expression analysis. Insect Science 23: 438–451.

Jakobs R., T.D. Gariepy and B.J. Sinclair. 2015. Adult plasticity of cold tolerance in a continental-temperate population of *Drosophila suzukii*. Journal of Insect Physiology 79: 1-9.

Jakobs R., B. Ahmadi, S. Houben, T.D. Gariepy and B.J. Sinclair. 2017. Cold tolerance of third-instar *Drosophila suzukii* larvae. Journal of Insect Physiology 96: 45-52.

Jensen K., T.N. Kristensen, L-H.L. Heckmann and J.G. Sørensen. 2017. Breeding and maintaining high-quality insects. In: Insects as Food and Feed: From Production to Consumption (editors Arnold van Huis and Jeff Tomberlin), Wageningen Academic Publishers.

Kang D.S., D.L. Denlinger and C. Sim. 2014. Suppression of allatotropin simulates reproductive diapause in the mosquito *Culex pipiens*. Journal of Insect Physiology 64: 48-53.

Kang D.S., M.A. Cotten, D.L. Denlinger and C. Sim. 2016. Comparative Transcriptomics Reveals Key Gene Expression Differences between Diapausing and Non-Diapausing Adults of *Culex pipiens*. PLoS ONE 11: e0154892.

Kaunisto S., L.V. Ferguson and B.J. Sinclair. 2016. Can we predict the effects of multiple stressors on insects in a changing climate? Current Opinion in Insect Science 17: 55-61.

Koštál V., J. Korbelová, R. Poupardin, M. Moos and P. Šimek. 2016. Arginine and proline applied as food additives stimulate high freeze tolerance in larvae of *Drosophila melanogaster*. Journal of Experimental Biology 219: 2538-2367.

Koštál V., J. Korbelová, T. Štětina, R. Poupardin, H. Colinet, H. Zahradníčková, I. Opekarová, M. Moos and P. Šimek. 2016. Physiological basis for low-temperature survival and storage of quiescent larvae of the fruit fly *Drosophila melanogaster*. Scientific Reports 6: 32346.

Kotwica-Rolinska J., L. Pivarciova, H. Vaneckova and D. Dolezel. 2017. The role of circadian clock genes in the photoperiodic timer of the linden bug *Pyrrhocoris apterus* during the nymphal stage. Physiological Entomology, DOI: 10.1111/phen.12197.

Knutson, A., A. Mukherjee, D.A. Hahn and K.M. Heinz. 2014. Biological control of giant salvinia (*Salvinia molesta*) in a temperate region: cold tolerance and low temperature oviposition of *Cyrtobagous salviniae*. BioControl. 59: 781-790.

Li Y., L. Zhang, Q. Zhang, H. Chen and D.L. Denlinger. 2014. Host diapause status and host diets augmented with cryoprotectants enhance cold hardiness in the parasitoid *Nasonia vitripennis*. Journal of Insect Physiology 70: 8-14.

Li Y., L. Zhang, H. Chen, V. Kostal, P. Simek, M. Moos and D.L. Denlinger. 2015. Shifts in metabolomic profiles of the parasitoid *Nasonia vitripennis* associated with elevated cold tolerance induced by the parasitoid's diapause, host diapause and host diet augmented with proline. Insect Biochemistry and Molecular Biology 63: 34-46.

Liu Y., Q. Zhang and D.L. Denlinger. 2015. Imidazole derivative KK-42 boosts pupal diapause incidence and delays diapause termination in several insect species. Journal of Insect Physiology 74: 38-44.

Liu Y., D.L. Denlinger and P.M. Piermarini. 2017. The diapause program impacts renal excretion and molecular expression of aquaporins in the northern house mosquito, *Culex pipiens*. Journal of Insect Physiology 98: 141-148.

Luypaert G., J. Witters, J. Van Huylenbroeck, M. Maes, J. De Riek and P. De Clercq. 2014. Temperature-dependent development of the broad mite *Polyphagotarsonemus latus* (Acari: Tarsonemidae) on *Rhododendron simsii*. Experimental and Applied Acarology 63: 389-400.

MacMillan H.A., L.V. Ferguson, A. Nicolai, A. Donini, J.F. Staples and B.J. Sinclair. 2015. Parallel ionoregulatory adjustments underlie phenotypic plasticity and evolution of *Drosophila* cold tolerance. Journal of Experimental Biology 218: 423-432.

MacMillan H.A., J.M. Knee, A.B. Dennis, H. Udaka, K.E. Marshall, T.J.S. Merritt and B.J. Sinclair. 2016. Cold acclimation wholly reorganises the *Drosophila melanogaster* transcriptome and metabolome. Scientific Reports 6: 28999.

Manenti T., V. Loeschcke, N.N. Moghadam and J.G. Sørensen. 2015. Phenotypic plasticity is not affected by experimental evolution in constant, predictable or unpredictable fluctuating thermal environments. Journal of Evolutionary Biology, 28: 2078–2087.

Meuti M.E., C.A. Short and D.L. Denlinger. 2015. Mom matters: diapause characteristics of *Culex pipiens-Cx. quinquefasciatus* (Diptera: Culicidae) hybrid mosquitoes. Journal of Medical Entomology 52: 131-137.

Meuti M.E., M. Stone, T. Ikeno and D.L. Denlinger. 2015. Functional circadian clock genes are essential for the overwintering diapause of the Northern house mosquito, *Culex pipiens*. Journal of Experimental Biology 218: 412-422.

Moraiti C.A., C.T. Nakas and N.T. Papadopoulos. 2017. Local adaptation, intrapopulation adult emergence patterns, sex and prolonged diapause regulate the rate of post-winter development in pupae of the European cherry fruit fly. Biological Journal of the Linnean Society 121: 282-293.

Mukai A. and S.G. Goto. 2016. The clock gene period is essential for the photoperiodic response in the jewel wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae). Applied Entomology and Zoology 51, 185-194.

Miyazaki Y., K. Tanaka, Y. Watari and S.G. Goto. 2016. Temperature cycle amplitude alters the adult eclosion time and expression pattern of the circadian clock gene period in the onion fly. Journal of Insect Physiology 86, 54-59.

Omura S., H. Numata and S.G. Goto. 2016. Circadian clock regulates photoperiodic responses governed by distinct output pathways in the bean bug, *Riptortus pedestris*. Biological Rhythm Research 47, 937-945**.**

Overgaard J. and H.A. MacMillan. 2017. The Integrative Physiology of Insect Chill Tolerance. Annual Review of Physiology 79: 187-208.

Owen E.L., J.S. Bale and S.A.L. Hayward. 2016. Establishment risk of the commercially imported bumblebee Bombus terrestris dalamtinus – can they survive UK winters? Apidologie 47: 66-75.

Pateman R.M., C.D. Thomas, S.A.L. Hayward and J.K. Hill. 2016. Macro- and micro-climate interactions drive variation in species' habitat associations. Glob. Change Biol. 22: 556-566.

Pivarciova L., H. Vaneckova, J. Provaznik, B.C. Wu, M. Pivarci, O. Peckova, O. Bazalova, S. Cada, P. Kment, J. Kotwica-Rolinska, and D. Dolezel. 2016. Unexpected geographic variability of the free running period in the linden bug *Pyrrhocoris apterus*, Journal of Biological Rhythms 31: 818-826.

Poupardin R., K. Schöttner, J. Korbelová, J. Provazník, D. Doležel, D. Pavlinic, V. Beneš and V. Koštál. 2015. Early transcriptional events linked to induction of diapause revealed by RNAseq in larvae of drosophilid fly, *Chymomyza costata*. BMC Genomics 16: 720.

Reynolds J.A., R. Bautista-Jimenez and D.L. Denlinger. 2016. Changes in histone acetylation as potential mediators of pupal diapause in the flesh fly, *Sarcophaga bullata*. Insect Biochemistry and Molecular Biology 76: 29-37.

Reynolds J.A., J.T. Peyton and D.L. Denlinger. 2017. Changes in microRNA abundance may regulate diapause in the flesh fly, *Sarcophaga bullata*. Insect Biochemistry and Molecular Biology 84: 1-14.

Rull J., E. Tadeo, R. Lasa and M. Aluja. 2016. The effect of winter length on survival and duration of dormancy of four sympatric species of *Rhagoletis* exploiting plants with different fruiting phenology. Bulletin of Entomological Research, 106: 818-826.

Salehipour-shirazi G., L.V. Ferguson and B.J. Sinclair. 2017. Does cold activate the *Drosophila melanogaster* immune system? Journal of Insect Physiology 96: 29-34.

Si F.L., Z.B. He and B. Chen. 2016. Cloning and expression profiling the heat shock protein DaHSP23 gene in the winter and summer diapause pupae of th onion maggot, *Delia antiqua* (Diptera: Anthomyiidae). Acta Entomologica Sinica 59: 402-410.

Sim C., D.S. Kang, S. Kim, X. Bai and D.L. Denlinger. 2015. Identification of FOXO targets that generate the diverse features of the diapause phenotype in the mosquito *Culex pipiens*. Proceedings of the National Academy of Sciences, USA 112: 3811-3816.

Sinclair B.J., L.E. Coello Alvarado, and L.V. Ferguson. 2015. An invitation to measure insect cold tolerance: Methods, approaches, and workflow. Journal of Thermal Biology 53: 180-197.

Sinclair B.J., K.E. Marshall, M.A. Sewell, D.L. Levesque, C.S. Willett, S. Slotsbo, Y. Dong, C.D.G. Harley, D.J. Marshall, B.S. Helmuth and R.B. Huey. 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? Ecology Letters 19: 1372-1385.

Slotsbo S., M.F. Schou, T.N. Kristensen, V. Loeschcke and J.G. Sørensen. 2016. Reversibility of developmental heat and cold plasticity is asymmetric and has long lasting consequences for adult thermal tolerance. Journal of Experimental Biology, 219: 2726-2732.

Sørensen J.G., M.F. Schou, T.N. Kristensen, and V. Loeschcke. 2016. Thermal fluctuations affect the transcriptome through mechanisms independent of average temperature. Scientific Reports, 6: 30975.

Stevenson T.J., M.E. Visser, W. Arnold, P. Barrett, S. Biello, A. Dawson, D.L. Denlinger, D. Dominoni, F.J. Ebling, S.Elton, N. Evans, H.M. Ferguson, R.G. Foster, M Hau, D.T. Haydon, D.G.Halerigg, P. Heideman, J.G.C. Hopcraft, N.N. Jonsson, N. Kronfeld-Schor, V. Kumar, G.A. Lincoln, R. MacLeod, S.A.M. Martin, M. Martinez-Bakker, R.J. Nelson, T. Reed, J.E. Robinson, D.Rock, W.J. Schwartz, I. Steffan-Dewenter, E. Tauber, S.J. Thackeray, C. Umstattter, T. Yoshimura and B. Helm. 2015. Disrupted seasonal biology impacts health, food security, and ecosystems. Proceedings of the Royal Society B 282: 20151453.

Teets N.M. and D.L. Denlinger. 2016. Quantitative phosphoproteomics reveals signaling mechanisms associated with rapid cold hardening in a chill-tolerant fly. Journal of Proteome Research 15: 2855-2862.

Terblanche J.S. 2014. Physiological performance of field-released insects Current Opinion in Insect Science 4: 60–66.

Terhzaz S., N.M. Teets, P. Cabrero, L. Henderson, M.G. Ritchie, R.J. Nachman, J.A.T. Dow, D.L. Denlinger and S.-A. Davies. 2015. The insect capa neuropeptides impact desiccation and cold tolerance Proceedings of the National Academy of Sciences, USA 112: 2882-2887. Selected as Editors' Choice in *Science Signaling* 8,ec54.

Toxopeus J., R. Jakobs, L.V. Ferguson, T.D. Gariepy and B.J. Sinclair. 2016. Reproductive arrest and stress resistance in winter-acclimated *Drosophila suzukii*. Journal of Insect Physiology 89: 37-51.

Urbanová V., O. Bazalová, H. Vanèćcková and D. Dolezel. 2016. Photoperiod regulates growth of male accessory glands through juvenile hormone signaling in the linden bug, *Pyrrhocoris apterus*. Insect Biochemistry and Molecular Biology 70: 184-190.

Van Damme V., N. Berkvens, R. Moerkens, E. Berckmoes, L. Wittemans, R. De Vis, H. Casteels, L. Tirry and P. De Clercq. 2015. Overwintering potential of the invasive leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) as a pest in greenhouse tomato production in Western Europe. Journal of Pest Science 88: 533-541.

Williams C.M., M. Watanabe, M. Guarracino, M.B. Ferraro, A.S. Edison, T.J. Morgan, A. Boroujerdi and D.A. Hahn. 2014. Cold adaptation shapes the robustness of metabolic networks in *Drosophila melanogaster*. Evolution. 68: 3505-3523.

Yang L., D.L. Denlinger and P.M. Piermarini. 2017. The diapause program impacts renal excretion and molecular expression of aquaporins in the northern house mosquito, Culex pipiens. Journal of Insect Physiology 98: 141-148.

Zhang Q., P.M. Piermarini, R.J. Nachman and D.L. Denlinger. 2014. Molecular identification and expression analysis of a diapause hormone receptor in the corn earworm, *Helicoverpa zea*. Peptides 53: 250-257.

Zhang Q., R.J. Nachman and D.L. Denlinger. 2015. Diapause hormone in the *Helicoverpa/Heliothis* complex: a review of gene expression, peptide structure and activity, analog and antagonist development, and the receptor. Peptides 72: 196-201.

Zhang Q., R.J. Nachman, K. Kaczmarek, K. Kierus, J. Zabrocki and D.L. Denlinger. 2015. Development of neuropeptide analogs capable of traversing the integument: a case study using diapause hormone analogs in *Helicoverpa zea*. Insect Biochemistry and Molecular Biology 67: 87-93.

ANNEX 1: List of participants

Third RCM on "Dormancy Management to Enable Mass-rearing and Increase Efficacy of Sterile Insects and Natural Enemies"

Vienna, Austria

29 May to 2 June 2017

3

BANGLADESH
Mr Mahbub Hasan
Department of Zoology
University of Rajshahi
Rajshahi 6205
Email: mmhbgd@yahoo.com

BELGIUM
Ms Astrid Bryon
Laboratory of Agrozoology
Department of Crop Protection, Faculty of Bioscience Engineering
Ghent University, Coupure Links 653
B-9000 Gent
Email: Astrid.Bryon@ugent.be

CANADA
Mr Brent Sinclair
University of Western Ontario
1151 Richmond St N
London, Ontario N6A 5B7
Email: bsincla7@uwo.ca

CHINA
Mr Yongcheng Dong
College of Plant Science and Technology
Huazhong Agricultural University
Wuhan, Hubei Province
Email: ccdyc@163.com

Mr Bin Chen
Chongqing Normal University
Institute of Entomology and Molecular Biology
No.37, Middle Daxuecheng Road
Huxi Town
Chongqing Shapingba District 401331
Email: binchen@cqnu.edu.cn

CZECH REPUBLIC
Mr David Dolezel
Institute of Entomology
Biology Centre ASCR
Branisovska 31
370 05 Ceske Budejovice
Email: david.dolezel@entu.cas.cz

DENMARK
Mr Kim Jensen
Aalborg University
Frederick Bajers Vej 7H
Aalborg East 9220
Email: kjensen@ncsu.edu

FRANCE
Mr Hervé Colinet
University of Rennes 1
263 AVE du General Leclerc
P.O. Box: CS 74205
35042 Rennes
Email: herve.colinet@univ-rennes1.fr

GREECE
Mr Nikolaos Papadopoulos
School of Agricultural Sciences
University of Thessaly
Fytokou Street
38446 Volos N. Ionia
Tel: 30 2421093285
Email: nikopap@uth.gr

Mr Nikos Kouloussis
Aristotle University of Thessaloniki
KEDEA Building
Tritis Septemvriou
Thessaloniki 54636
Email: nikoul@agro.auth.gr

REPUBLIC OF KOREA
Mr Jun Seok Lee
Institute for Bioresouces
910, 27-34 GwanonRo
GURI 471-841
Email: jslee@k-insect.com

SOUTH AFRICA
Mr Jonathan Terblanche
Stellenbosch University
Department of Conservation Ecology & Entomology
APE Lab
Private Bag X1
MATIELAND 7602
Email: jst@sun.ac.za

UNITED KINGDOM
Mr Scott Hayward
University of Birmingham
Edgbaston
Birmingham, West Midlands B15 2TT
Email: s.a.hayward@bham.ac.uk

UNITED STATES OF AMERICA
Mr David L. Denlinger
Ohio State Univ.Dept Entomol.
300 Aronoff Lab.
318 West 12th Av.
43210 Columbus, OH
Email: denlinger.1@osu.edu

Mr Daniel Hahn
Department of Entomology and Nematology
University of Florida
P.O. Box 110620
32611-0620 Gainesville, FL
Email: dahahn@ufl.edu

Mr Joseph Rinehart
USDA-ARS
RRVARC
1605 Albrecht Blvd
Fargo, ND 58102-2765
Email: Joseph.Rinehart@ARS.USDA.GOV

OBSERVERS

CHINA
Mr Youjin Hao
Institute of Entomology and Molecular Biology
College of Life Sciences
Chongqing Normal University
University Town
CHONGQING 401331
Email: haoyoujin@hotmail.com

REPUBLIC OF KOREA
Ms Eunhye Ham
Research Project Planning Division
Institute for Bioresources
Osangkinsect Co., Ltd.
805, 27(Inchang-dong) 34 Geonwon-daero
GURI-SI, GG
Email: ehham@k-insect.com

SOUTH AFRICA
Mr Bev Grieshaber
Stellenbosch University
Department of Conservation Ecology & Entomology, APE LAB
Private Bag X1
7602 MATIELAND
Email: beverleyjg@gmail.com

Ms **Daleen Stenekamp**
Hortgro Science
P.O. Box 12789
DIE BOORD 7613
Email: daleen@hortgro.co.za

UNITED STATES OF AMERICA
Ms Leigh Boardman
Department of Entomology and Nematology
University of Florida
P.O. Box 110620
GAINESVILLE FL 32611-0620
Email: lboardman@ufl.edu

ANNEX 2: Agenda

THIRD FAO/IAEA RESEARCH COORDINATION MEETING ON

Dormancy Management to Enable Mass-rearing and Increase Efficacy of Sterile Insects and Natural Enemies

29 May to 2 June 2017, Vienna, Austria

Vienna International Centre (IAEA Headquarters), Building M - Room M5

AGENDA

MONDAY, 29 MAY 2017

08:00 – 08:45	**Identification and registration at VIC Gate (next to subway station U1)**
08:45 – 09:00	**Rui Cardoso Pereira:** Welcome statement, goals of the CRP and the meeting, agenda and administrative issues
	SESSION I: Manipulating Dormancy Responses (Chairperson: Nikos Papadopoulos)
09:00 – 09:30	Dave Denlinger: **New players in an old game: The search for microRNAs as regulators of insect diapause**
09:30 – 10:00	**Dan Hahn:** Describing the diapause-preparatory proteome of the beetle *Colaphellus bowringi* and identifying candidates affecting lipid accumulation using isobaric tags for mass spectrometry-based proteome quantification (iTRAQ)
10:00 – 10:30	**Dong Yongcheng:** Proteomic analysis of diapause transition in *Bactrocera minax* by 20-hydroxyecdysone application
COFFEE BREAK	**(Bank issues)**
11:00 – 11:30	**Juan Rull:** Prolonged dormancy as a life-history strategy among specialized herbivorous Rhagoletis fruit flies from high elevation environments with acyclic climatic variability
11:30 – 12:00	**Patrick De Clercq:** The overwintering ability of two dipteran insects: *Drosophila suzukii* and *Hermetia illucens*
LUNCH	
13:00 – 13:30	**Martin Schebeck:** Genomic background of diapause phenotypes in European *Ips typographus*
13:30 – 14:00	Mahbub Hassan: **Mass-rearing and control strategies for Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae): Minimizing their dormancies**

14:00 – 14:30 **Shin Goto:** Physiological and molecular mechanisms underlying embryonic diapause in the band-legged ground cricket *Dianemobius nigrofasciatus*

14:30 – 15:00 **Nick Howe:** Considerations of dormancy in rearing a novel commercial pollinator species: getting *Osmia bicornis* 'off the shelf'

COFFEE BREAK

15:30 – 16:00 **Astrid Bryon:** Carotenoids and diapause in the two-spotted spider mite

SESSION II: Low Temperature Biology (Chairperson: Juan Rull)

16:00 – 16:30 **Brent Sinclair:** Progress in understanding freeze tolerance and metabolic suppression in dormant insects

16:30 – 17:00 **Johannes Overgaard:** The integrative physiology of insect chill tolerance

TUESDAY, 30 MAY 2017

08:30 – 09:00 Jan Rozsypal: **Survival of internal ice formation in freeze avoiding insects: role of inoculation by external ice**

09:00 – 09:30 **Minette Karsten:** Mechanisms underlying low temperature performance and their potential for manipulating field performance

09:30 – 10:00 **Nikos Papadopoulos:** Effect of pre-chilling interval on diapause termination, and prolonged dormancy induction in pupae and a first effort to explore cold tolerance of both pupae and adult *Rhagoletis cerasi* (Diptera: Tephritidaea)

COFFEE BREAK

10:30 – 11:00 **Hervé Colinet:** Assessment of basal thermal tolerance of *Drosophila suzukii* and optimizing storage conditions under constant and/or fluctuating cold conditions

11:00 – 11:30 **Christos Gerofotis:** Effect of temperature and photoperiod on diapause termination, longevity and morphology of *Eurytoma plotnikovi*

11:30 – 12:00 **Eunhye Ham:** Cold tolerance and the effect of irradiation of the pine sawyer beetle, *Monochamus saltuarius* and the Japanese pine sawyer beetle *Monochamus alternates* (Coleoptera: Cerambycidae)

LUNCH

13:00 – 13:30 **Lauren Des Marteaux:** Effects of cold acclimation on tissue structure and active transport in the ionoregulatory tissues of adult *Gryllus pennsylvanicus* field crickets

13:30 – 14:00 **Jesper Sørensen:** Optimizing performance of biological control agents using diets, temperatures and behavioral traits

14:00 – 14:30 **Kendra Greenlee:** Improving long- and short-term storage to increase pollinator quality

14:30 – 15:00	**Mariana Viscarret:** Storage and maternal effect on *Trichogramma nerudai*

COFFEE BREAK

	SESSION III: General Discussion (Chairperson: Rui Cardoso Pereira)
15:30 – 16:30	General Discussion
16:30 – 17:00	Selection of Working Groups
18:00	**GROUP DINNER AT SCHWEIZERHAUS**

WEDNESDAY, 31 MAY 2017

	SESSION IV: Results Obtained and Research Gaps (Chairperson: Rui Cardoso Pereira and Group Leaders) (Rooms M5 and MOE26)
08:30 – 10:00	Working Groups: Results obtained and review of research gaps that need to be addressed

COFFEE BREAK

10:30 – 12:00	Working Groups: Continued review of results obtained and research gaps that need to be addressed

LUNCH

13:00 – 15:00	Working Groups: Continued review of results obtained and research gaps that need to be addressed

COFFEE BREAK

15:30 – 17:00	General Discussion: Review of results obtained, and research gaps that need to be addressed

THURSDAY, 1 JUNE 2017

	SESSION V: Review of the Individual Proposals (Chairperson: Rui Cardoso Pereira and Group Leaders) (Rooms M5 and MOE26)
08:30 – 10:00	Working Groups: Review of individual research proposals for the different working areas

COFFEE BREAK

10:30 – 12:00	Working Groups: Review of individual research proposals for the different working areas

LUNCH

13:00 – 15:00	Working Groups: Review of individual research proposals for the different working areas

COFFEE BREAK

15:30 – 17:00	General Discussion: Review of individual research proposals for the different working areas

FRIDAY, 2 JUNE 2017

	SESSION VI: RCM Report (Chairperson: Rui Cardoso Pereira and Group Leaders)
08:30 – 10:00	Review and adjustment of the logical framework

COFFEE BREAK

10:30 – 12:00	Agreement on content of RCM report, and drafting and compiling of RCM report

LUNCH

13:00 – 15:00	Agreement on CRP final publication, on information exchange mechanisms, on location of Final RCM, and closure of the RCM