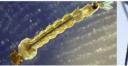


BOOK of ABSTRACT



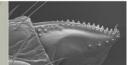


Trento, 7-9 November 2016









Monday 7th November

09:30 - 10:30	Participants registration
10:30 - 11:00	Opening ceremony
11:00 – 11:50	Peter Hudson (Keynote talk):
	What are the challenges for the future study of vector borne diseases? Some
	thoughts about patterns, processes and controls
Session 1. The	Mediterranean fruit fly: a model of a cosmopolitan invader
Chair: Francesca Scolari	
12:00 – 12:20	Hannes Schuler
	The infection dynamics of Wolbachia between native and invasive Rhagoletis
	fruit flies in Europe
12:20 – 12:40	Anna Malacrida
	Chemoreception as an adaptive trait in the highly Invasive fruitfly Ceratitis
	capitata (Diptera, Tephritidae)
12:40 – 13:00	Francesca Scolari
	Larval diet impacts the composition of male pheromone blend in the
	Mediterranean fruit fly, Ceratitis capitata
	Lunch
	sive Alien Specie: miscellanea
Chair: Valerio I	
14:30 – 14:50	Valerio Mazzoni
	Potential use of vibrational signals to manage the brown marmorated stink
	bug, Halyomorpha halys, a new invasive agricultural pest
14:50 – 15:10	'
	Massive yet massively underestimated costs of invasive insects
15:10 – 15:30	Rupert Houghton
	Optimising invasive crayfish management by incorporating an understanding of
	population dynamics and their Interaction with traditional control methods in
	different seasons
15.30 – 15:50	Fabrizio Lombardo
15.50 16.10	Unraveling the olfactory repertoire of the tiger mosquito Aedes albopictus
15.50 – 16:10	Alekos Simoni
10.10 10.10	Genetic control of vector-borne disease by gene drive systems
16:10 – 16:40	Coffee break







Session 3. The invasion of the Asian hornet, Vespa velutina, and its impact on honey bees	
Chair: Alessandro Cini	
16:40 - 17:00	Alessandro Cini
	Contributing to the knowledge and management of the invasive hornet Vespa
	velutina: early warning tools, sexual communication and competition with
	native hornets
17:00 – 17:20	Morgane Barbet-Massin
	The invasion of the Asian hornet (Vespa velutina nigrithorax) in France and
	Europe: estimating the different economic costs
17:20 – 17:40	Alice Fournier
	Novel prediction of Asian hornet invasion range and impact
17:40 – 18:00	Sandro Bertolino
	Natural and human-mediated diffusion of Vespa velutina in Italy and
	forecasting short term spread to improve management activities
18:00 - 18:50	Micheal Turelli (Keynote talk):
	Spread of introduced Wolbachia in natural insect populations: robust
	predictions relevant to dengue (and possibly Zika) control
19:00 -	Welcome cocktail

Tuesday 8th November

Session 4. Studies on the ecology of mosquito and mosquito-borne pathogens	
Chair: Alessia Melegaro	
08:30 - 09:00	Hervé Zeller (Keynote talk):
	Are mosquito borne diseases transmitted by invasive Aedes mosquitoes an
	emerging threat for Europe?
09:00 - 09:20	Giuliano Gasperi
	Global Invasion of Aedes albopictus as a vector of arbovirus: what consequences
	for disease outbreaks?
09:20 - 09:40	Ruth Müller
	Cold-hardiness of the Asian tiger mosquito Aedes albopictus, a vector of
	multiple viral and parasitic pathogens
09:40 - 10:00	Meghnath Dhimal
	Climate change and expansion of mosquito-borne viral diseases in the Hindu
	Kush Himalayan region
10:00 - 10:20	Lino Ometto
	Codon usage and phylogenetic analyses elucidate the recent evolutionary
	history of Zika virus
10:20 - 10:40	Maria Vittoria Mancini
	Insights into the bacterial microbiota components of vectors circulating in
	Marche region, Italy







10:40 - 11:10	Coffee break	
Session 5. Tools and novel methods for mosquito surveillance		
Chair: Fabrizio Montarsi		
11:10 – 11:30	Laura Vavassori	
	Surveillance of invasive mosquitoes in Switzerland	
11:30 – 11:50	Andrea Mosca	
	Exotic mosquito surveys in Piedmont	
11:50 – 12:10	Marco Ballardini	
	Invasive mosquitoes keep spreading in Northern Italy: first report of <i>Aedes</i>	
	koreicus in the Liguria region	
12:10 – 12:30	Beniamino Caputo	
	Development of Novel Non-Conventional Approaches to Monitor <i>Aedes</i>	
	albopictus Abundance and Biting Rates	
12:30 – 12:50	Mattia Manica	
	Estimating Aedes albopictus biting females by ovitrap surveillance data in an	
	area at high-risk of exotic arbovirus introduction	
12:50 – 14:20	Lunch	
	quito susceptibility and resistance to insecticides	
	Alexandre Baldacchino	
14:20 – 15:00	Alessandra della Torre (Keynote talk):	
	Targeting Aedes albopictus in a metropolitan European area	
15:00 – 15:20	Friederike Reuss	
	Insecticides and essential oils against the Asian bush mosquito	
15:20 – 15:40	Mariangela Bonizzoni	
	Resistance to pyrethroids in <i>Aedes albopictus</i> : results from a multi-country	
	survey	
15:40 – 16:00	Verena Pichler	
	Susceptibility of Italian Aedes albopictus and Culex pipiens populations to	
	insecticides most widely used in interventions against adult mosquitoes	
16:00 – 16:30	Coffee break	







Session 7. Lexem project results on mosquito biology, modelling and control	
Chair: Rosà Roberto	
16:30 - 17:10	Stefano Merler (Keynote talk):
	Population dynamics of endemic mosquitoes species and epidemiological
	implications in Northern Italy
17:10 - 17:30	Gioia Capelli
	Determination of ecological parameters of the invasive mosquito species Aedes
	albopictus and Aedes koreicus in a mountainous environment of Northeastern
	Italy and experimental and applied control
17:30 – 17:50	Markus Metz
	Potential distribution of invasive species in Italy
17:50 – 18:10	Filippo Trentini
	Preventing the risk of Chikungunya outbreaks through vector control programs
	in temperate climate countries: a cost-utility analysis in the epidemic scenario
	of Emilia Romagna
19:00 – 20.00	Visit Science Museum (MUSE) in Trento
20:00 -	Social dinner at MUSE

Wednesday 9th November

Session 8. Lexem project results on Drosophila suzukii biology, modelling and control	
Chair: Andrea Pugliese	
08:30 - 09:20	Claudio Ioriatti (Keynote talk):
	Current status of the <i>Drosophila suzukii</i> management in Trentino (Italy):
	research achievements and perspectives for sustainable control
09:20 - 09:40	Marco Valerio Rossi-Stacconi
	Biological control of spotted wing <i>Drosophila</i> by using Italian resident
	parasitoids: laboratory and field experiences
09:40 - 10:00	Andrea Pugliese
	Models of <i>Drosophila suzukii</i> population dynamics
10:00 - 10:20	Emanuele Dal Fava
	Cost-benefit analysis for controlling Drosophila suzukii spread and infestation
	of soft fruits in Northern Italy







Session 9. Exploring the genetic basis of Drosophila suzukii control		
Chair: Omar Rota-Stabelli		
10:20 - 10:40	Fabiana Sassù	
	Development of the sterile insect technique to manage confined populations of	
	Drosophila suzukii	
10:40 - 11:00	Francesco Drago	
	Phylogenetic incongruences indicate a complex evolutionary scenario for the	
	bio-control agent Wolbachia	
11:00 – 11:30	Coffee break	
11:30 - 11:50	Omar Rota-Stabelli	
	Genetic, evolutionary, symbiotic and phenotypic variations between European	
	and American strains of <i>Drosophila suzukii</i> : implications for biocontrol and	
	management studies	
11:50 - 12:10	Gabriella Tait	
	Reconstructing the colonization history and the population dynamic of	
	Drosophila suzukii in Italy	
12:10 – 12:30	Maria Cristina Crava	
	Transcriptome profiling of adult <i>Drosophila suzukii</i> antennae	
12:30 – 14:00	Lunch	
	dies on the biology and ecology of Drosophila suzukii	
Chair: Gianfrai		
14:00 – 14:20	Lorenzo Tonina	
	The role of wild hosts, natural habitat and low temperature to promote	
	Drosophila suzukii invasiveness in North-East Italy	
14:20 - 14:40	Virginia Belloni	
	Comparative study on ammonia and urea tolerance in Drosophila	
	melanogaster and Drosophila suzukii	
14:40 – 15:00	Baláz Kiss	
	Monitoring of invasive arthropods in highway rest areas in Hungary	
15:00 – 15:50	Vaughn Walton (Keynote talk):	
	Advances in the knowledge of <i>Drosophila suzukii</i> physiology and ecology	
15:50 – 16.00	Closing ceremony	





Trento, 7-9 November 2016



Summary

What are the challenges for the future study of vector borne diseases?
The Infection dynamics of Wolbachia between native and invasive Rhagoletis fruit flies in Europe
Chemoreception as an adaptive trait in the highly invasive fruitfly Ceratitis capitata (Diptera, Tephritidae) 10
Larval diet impacts the composition of male pheromone blend in the Mediterranean fruit fly, <i>Ceratitis</i> capitata
Potential use of vibrational signals to manage the Brown Marmorated Stink Bug, <i>Halyomorpha halys</i> , a new invasive agricultural pest
Massive yet massively underestimated costs of invasive insects
Optimising invasive crayfish management by incorporating an understanding of population dynamics and their interaction with traditional control methods in different seasons
Unraveling the olfactory repertoire of the tiger mosquito Aedes albopictus
Genetic control of vector-borne disease by gene drive systems
Contributing to the knowledge and management of the invasive hornet <i>Vespa velutina</i> : early warning tools, sexual communication and competition with native hornets
The invasion of the Asian hornet (<i>Vespa velutina nigrithorax</i>) in France and Europe: estimating the different economic costs
Novel prediction of Asian hornet invasion range and impact
Natural and human-mediated diffusion of <i>Vespa velutina</i> in Italy and forecasting short term spread to improve management activities
Spread of introduced <i>Wolbachia</i> in natural insect populations: robust predictions relevant to dengue (and possibly Zika) control
Are mosquito borne diseases transmitted by invasive Aedes mosquitoes an emerging threat for Europe?. 22
Global invasion of Aedes albopictus as a vector of arbovirus: what consequences for disease outbreaks? 23
Cold-hardiness of the Asian tiger mosquito <i>Aedes albopictus</i> , a vector of multiple viral and parasitic pathogens
Climate change and expansion of mosquito-borne viral diseases in the Hindu Kush Himalayan region 25
Codon usage and phylogenetic analyses elucidate the recent evolutionary history of Zika virus 26
Insights into the bacterial microbiota components of vectors circulating in Marche region, Italy
Surveillance of invasive mosquitoes in Switzerland
Exotic mosquito surveys in Piedmont
Invasive mosquitoes keep spreading in Northern Italy: first report of <i>Aedes koreicus in</i> the Liguria region . 30
Development of novel non-conventional approaches to monitor <i>Aedes albopictus</i> abundance and biting rates







Estimating Aedes albopictus biting females by ovitrap surveillance data in an area at high-risk of exotic	22
arbovirus introduction	
Targeting Aedes albopictus in a metropolitan European area	
nsecticides and essential oils against the Asian bush mosquito	
Resistance to pyrethroids in <i>Aedes albopictus</i> : results from a multi-country survey	35
Susceptibility of Italian Aedes albopictus and Culex pipiens populations to insecticides most widely used interventions against adult mosquitoes	
Population dynamics of endemic mosquitoes species and epidemiological implications in Northern Italy .	37
Determination of ecological parameters of the invasive mosquito species <i>Aedes albopictus</i> and <i>Aedes koreicus</i> in a mountainous environment of Northeastern Italy and experimental and applied control	38
Potential distribution of invasive species in Italy	39
Preventing the risk of Chikungunya outbreaks through vector control programs in temperate climate countries: a cost-utility analysis in the epidemic scenario of Emilia Romagna	40
Current status of the <i>Drosophila suzukii</i> management in Trentino (Italy), research achievements and perspectives for sustainable control	41
Biological control of spotted wing Drosophila by using Italian resident parasitoids: laboratory and field experiences	42
Models of <i>Drosophila suzukii</i> population dynamics	43
Cost-benefit analysis for controlling <i>Drosophila suzukii</i> spread and infestation of soft fruits in Northern It	-
Development of the sterile insect technique to manage confined populations of <i>Drosophila suzukii</i>	
Phylogenetic incongruences indicate a complex evolutionary scenario for the bio-control agent <i>Wolbach</i>	
Genetic, evolutionary, symbiotic, and phenotypic variations between European and American strains of suzukii: implications for biocontrol and management studies	
Reconstruction the colonization history and the population dynamics of Drosophila suzukii in Italy	48
Transcriptome profiling of adult Drosophila suzukii antennae	49
The role of wild hosts, natural habitat and low temperature to promote <i>Drosophila suzukii</i> invasiveness i North-East Italy	
Comparative study on ammonia and urea tolerance in Drosophila melanogaster and Drosophila suzukii	51
Monitoring of invasive arthropods in highway rest areas in Hungary	52
Advances in the knowledge of <i>D. suzukii</i> physiology and ecology	53







KEYNOTE TALK

What are the challenges for the future study of vector borne diseases?

Peter J. Hudson

Center for Infectious Disease Dynamics, Penn State University, USA

Over the past few years, the developed world has experienced a series of invasions by vector borne infections such as Zika, Chikungunya and West Nile. In many respects we did not predict this and we need to think about what we have learned from these experiences and how this should help our research going forward. Rather than look back at what has happened I shall try and throw out some ideas about what I see as some of the challenges for vector borne disease research and application in the future. First, this will include a discussion of transmission - for example, one of the astonishing findings about Zika are the multiple routes of transmission of this virus and raises the question "With vector borne viral diseases are we convinced that we should focus solely on vector borne transmission?" Second, recent studies examining the response of vectors and their diseases to temperature variation show us many non-linear responses and to my mind make us think more about trait based modelling approaches. Finally many infections exhibit a wide range of responses in human hosts that can range from mild headaches through to death-inducing fevers - we focus on this being the result of a single infection and yet we suspect that pathogens interact and one pathogen could change the likelihood of transmission and the disease symptoms of another - is it time for a community ecology approach to transmission?

CONTACT MAIL: pjh18@psu.edu







The Infection dynamics of *Wolbachia* between native and invasive *Rhagoletis* fruit flies in Europe

Hannes Schuler¹, Wolfgang Arthofer², Christian Stauffer¹

¹Institute of Forest Entomology, Forest Pathology and Forest Protection, Department of Forest & Soil Sciences, Boku, University of Natural Resources and Life Sciences, Vienna, Austria ²Molecular Ecology Group, University of Innsbruck, Austria

Wolbachia is a widespread endosymbiotic bacteria infecting different arthropod species. Key factor of its successful lifestyle is the ability to manipulate the reproduction of its host and promote vertical transmission. However, incongruence between Wolbachia and host phylogenies showed the absence of long-term Wolbachia-host coevolution and that the bacteria can be transmitted horizontally among species. Key factor for horizontal transmission between two different species is the close physical contact between donor and recipient. The ecological overlap of two phytophagous species attacking the same host can provide horizontal transmission paths. Therefore, the colonization of new habitats of invasive species and the interaction with cooccurring native species can have effects on their endosymbiont community. The genus Rhagoletis comprises important agricultural pests with a broad geographic distribution and different host plant associations. The eastern cherry fruit fly Rhagoletis cingulata infests the fruits of several cherry species in its native range in North America. R. cingulata was recently introduced to Europe where it co-infests cherries with the native European cherry fruit fly Rhagoletis cerasi. Here we characterize the Wolbachia infection of native and invasive cherry-attacking Rhagoletis fruit flies and show multiple horizontal transmission events of Wolbachia between the two species. We describe the infection dynamics of the newly acquired Wolbachia strain and discuss its potential influence on the evolution and ecology of its host.

CONTACT MAIL: hannes.schuler@boku.ac.at







Chemoreception as an adaptive trait in the highly invasive fruitfly *Ceratitis capitata* (Diptera, Tephritidae)

Anna R. Malacrida, Ludvik M. Gomulski, Francesca Scolari, Grazia Savini, Giuliano Gasperi

Department of Biology and Biotechnology, University of Pavia, 27100 Pavia, Italy

The highly invasive agricultural insect pest, Ceratitis capitata (Diptera: Tephritidae) is the most thoroughly studied tephritid fruit fly species, and it has become a model for the analysis of fruit fly invasions. It is an opportunistic phytophagous species whose survival and dispersion is tightly dependent on its interactions with different environments where the plasticity of its chemoreception plays a fundamental role. The chemosensory repertoire of the medfly genome has been annotated, and a comparative analysis with the chemosensory repertoires of Drosophila melanogaster and Musca domestica revealed that the medfly is more similar to Drosophila than Musca, perhaps because its ecology is more similar, despite being phylogenetically more basal. Olfaction in the medfly plays a key role in regulating essential reproductive behaviours such as the detection of pheromones during the recognition and location of mates and ovipostion sites. We are characterizing at the genetic, molecular, functional and structural levels the chemosensory components that regulate the complex pheromone communication system. These analyses are an important step for the interpretation of the biology of the species and consequently its invasive potential. In addition, the identification of the components of the molecular machinery involved in the binding and recognition of odours and pheromones is essential for the development of powerful species- and sex-specific attractants.

CONTACT MAIL: malacrid@unipv.it





Trento, 7-9 November 2016



Larval diet impacts the composition of male pheromone blend in the Mediterranean fruit fly, *Ceratitis capitata*

<u>Francesca Scolari</u>¹, Daniele Merli², Grazia Savini¹, Anna R. Malacrida¹, Giuliano Gasperi¹

The Mediterranean fruit fly (medfly), Ceratitis capitata (Diptera: Tephritidae), is a worldwide pest of fruit and crops. Medfly males emit a complex pheromone blend either to attract other males, which aggregate in small leks on trees or bushes, and females for mating. The wide host range displayed by this species allows to hypothesize that pheromone blend can vary according to the different host plants. This is supported by the fact that the pheromone of laboratory and wild-type males differs both qualitatively and quantitatively. In this study, we tested whether the composition of medfly pheromone is influenced by the diet experienced during the larval stage. Medflies from the well established Ispra laboratory strain, whose genome has been recently sequenced, were separately reared on different larval media, namely two artificial diets (branbased, and dehydrated carrot powder-based, respectively), and three fresh fruits (oranges -Valencia Late and Navel varieties, and apricots). Flies weresexed at emergence and maintained as virgins until sexual maturity. The volatile emissions of mature males were collected by Headspace Solid Phase Microextraction (HSSPME) and subsequently analyzed by Gas Chromatography coupled with Mass Spectrometry (GC/MS). Our results suggest that pheromone emissions vary both qualitatively and quantitatively across the different diets. Interestingly, major medfly pheromone components such as (E,E)-alpha-farnesene and geranyl acetate were found to be consistently present, despite the different larval diet. In addition, the pheromone emitted by one month-old males was also tested, showing that signaling capacity was not impaired. A better understanding of the effects of different pre-adult rearing environments on adult mating behavior and mate recognition signals will contribute unravelling medfly chemosensory biology, enable improved mass rearing, and help developing novel strategies for population control.

CONTACT MAIL: francesca.scolari@unipv.it

¹Dipartimento di Biologia e Biotecnologie, Università di Pavia, 27100 Pavia, Italy ²Dipartimento di Chimica, Università di Pavia, 27100, Pavia, Italy





Trento, 7-9 November 2016









Potential use of vibrational signals to manage the Brown Marmorated Stink Bug, Halyomorpha halys, a new invasive agricultural pest

<u>Valerio Mazzoni</u>¹, Gianfranco Anfora¹, Marco Valerio Rossi-Stacconi¹, Jernej Polajnar^{1,2}, Marta Baldini³, Roberto Guidetti³, Lara Maistrello³

The Brown Marmorated Stink Bug (BMSB), Halyomorpha halys (Hemiptera: Pentatomidae) is a polyphagous insect that can cause severe economic damage on different crops. In Italy this species was first recorded in the region Emilia Romagna in 2012 and in few years it spread in most of the Northern and part of the Central Italian regions. As well as in other stink bugs the mid-short range mating communication is based on the exchange of vibrational signals (duet) between sexes that is the key of pair formation, courtship and copula. During the duet females are stationary and males actively search on the plant. In other pentatomids the directionality of the searching in males, given by female attractive signals, has been demonstrated. We hypothesize that the same principle works also in BMSB and thus it would be possible to use the female calling signal to attract males towards precise source sites (i.e. artificial shakers). If confirmed, this would represent a potential implementation to the traps commonly used for BMSB monitoring, by providing an additional stimulus to enlarge the amount of captured bugs. To assess whether the female calling signal could be employed to capture males we performed different bioassays with males stimulated with either natural or synthetic vibrational signals, in different scenarios (i.e., net cages, round arenas, potted plants). Vibrational signals were recorded with laser vibrometer and a video analysis software was used to study the behavioural responses. Our experiments demonstrated the attractive effect of the female signal in all performed tests. On average, more than 50% of the tested males were attracted to vibrating sources. In addition, we also found a significant increase of walking speed, walked distance and time spent in stimulated areas. These results show promise for developing more efficient trapping techniques against H. halys in both agricultural and urban environments.

CONTACT MAIL: valerio.mazzoni@fmach.it

¹Fondazione Edmund Mach, San Michele All'Adige, Trento, Italy

²National Institute of Biology, Ljubljana, Slovenia

³Università degli Studi di Modena e Reggio Emilia, Reggio Emilia, Italy





Trento, 7-9 November 2016



Massive yet massively underestimated costs of invasive insects

<u>Franck Courchamp</u>^{1,2,3}, Corey J. A. Bradshaw^{1,4}, Boris Leroy^{1,5}, Céline Bellard^{1,6}, Céline Albert¹, David Roiz⁷, Morgane Barbet-Massin¹, Alice Fournier¹, Jean-Michel Salles⁸, Frédéric Simard⁷

¹Ecologie, Systématique et Evolution, Université Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, 91400, Orsay Cedex, France

²Department of Ecology and Evolutionary Biology, University of California, 621 Young Drive South, Los Angeles, 90095-1606, USA

³Center for Tropical Research, Institute of the Environment and Sustainability, La Kretz Hall, University of California Los Angeles, CA 90095, USA

⁴School of Biological Sciences, The University of Adelaide, Adelaide, South Australia 5005, Australia ⁵UMR Biologie des Organismes et des Ecosystèmes Aquatiques, Département Milieux et Peuplements Aquatiques, Muséum National d'Histoire Naturelle, 43 rue Cuvier 75005 Paris, France ⁶Department of Genetics, Evolution and Environment, Centre for Biodiversity and Environment and Research, University College London, London WC1E 6BT, United Kingdom

⁷MIVEGEC, Université de Montpellier-IRD224-CNRS5290, 911 Avenue Agropolis, 34394 Montpellier, France

⁸Laboratoire Montpellierain d'Économie Théorique et Appliquée, UMR LAMETA Campus, INRA-SupAgro, Bat 26, 2 place Viala, 34060 Montpellier Cedex 2, France

Insects have for millennia presented human society with some of its greatest development challenges by spreading diseases, consuming crops and damaging infrastructure. Despite the massive human and financial toll of invasive insects, cost estimates of their impacts remain sporadic, spatially incomplete and of questionable quality. We compiled the most comprehensive database of economic costs of invasive insects, expressing historical estimates in annual 2014-equivalent US dollars. Taking all reported goods and services estimates, invasive insects cost a minimum of US\$70.0 billion year-1 globally, while global health costs directly attributable to invasive insects exceed US\$6.9 billion year-1. Total costs rise as the number of estimates increases, such that there is an order of magnitude increase for an additional 5-19 estimates per region of the world. A lack of dedicated studies implies gross underestimation of global costs. As climate change, rising human population densities and intensifying international trade will allow some of the costliest insects to spread into new areas, substantial savings could be achieved by increasing surveillance, containment and public awareness.

CONTACT MAIL: franck.courchamp@u-psud.fr





ecology, modelling and control of their economic impact and public health implicati Trento, 7-9 November 2016



Optimising invasive crayfish management by incorporating an understanding of population dynamics and their interaction with traditional control methods in different seasons

Rupert Houghton¹, Doerthe Tetzlaff², Collin Bean³, Robert Laughton⁴, Xavier Lambin⁵

There is an apparent reluctance to commit to long term population control of invasive crayfish populations such as the signal crayfish (Pacifastacus leniusculus), in spite of the known ecological and economic impacts and perhaps due to a perceived lack of success. Notable successful removal studies have been conducted for extended periods of time, using a minimum of two methods. However the majority of previous failed control efforts have typically made use of only a single removal method, applied without consideration of their seasonal variation in impact on the population dynamics. Signal crayfish are cannibalistic but this behaviour has not previously been considered as a possible density-dependent regulatory dynamic. We investigated whether this was the case in a small population with a varying density gradient across the stream length. I also conducted research to quantify the demographic selectivity and efficacy of six different traditional removal methods in spring, summer and autumn, whilst simultaneously performing a capture mark-recapture study in order to quantify the abundance of available crayfish in each demographic class. This resulted in harvest parameters that reflect the proportion of each demographic class removed per unit effort of each respective method in each seasonal period. Crayfish size and density both influenced cannibalism probability. Several different but related density metrics explained cannibalism incidence equally well but hinted that the density of small crayfish was the key driver of conspecific predation. We suggest that cannibalism might act to stabilise population growth by putting a cap on juvenile densities, which has important implications for management. After constructing and modelling a signal crayfish life cycle, I simulated the impacts of various seasonal combinations of removal methods on invasive crayfish population dynamics. Thus a seasonal combination of methods can be established that optimally decreases population growth rate. Additionally, this seasonally optimal eradication strategy can be compared with similar management plans that also incorporate male sterilisation, in order to establish the most cost effective long-term population control.

CONTACT MAIL: rupert.houghton@abdn.ac.uk

¹School of Biological Science, University of Aberdeen, Aberdeen, Aberdeenshire, United Kingdom

²School of Geosciences, University of Aberdeen, Aberdeen, Aberdeenshire, United Kingdom

³Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, Strathclyde, United Kingdom

⁴Findhorn, Nairn and Lossie Fisheries Trust, Moray, United Kingdom

⁵School of Biological Science, University of Aberdeen, Aberdeen, Aberdeenshire, United Kingdom





Trento, 7-9 November 2016



Unraveling the olfactory repertoire of the tiger mosquito Aedes albopictus

<u>Fabrizio Lombardo</u>¹, Marco Salvemini², Carmine Fiorillo¹, Tony Nolan³, José M. Ribeiro⁴, Bruno Arcà¹

The Asian tiger mosquito *Aedes albopictus* is a highly invasive species and competent vector of several arboviruses (e.g. dengue, chikungunya, Zika). Complex mosquito behaviours like host seeking, feeding, mating or oviposition rely on sensory functions carried out by olfactory neurons (ONs) localized mainly on antennae, mouthparts and maxillary palps. Typically, volatile odorants cross the cuticle and through Odorant Binding Proteins (OBPs) reach specific Odorant Receptors (ORs) on dendritic membranes of ONs. In order to characterize the main *Ae. albopictus* olfactory gene families we analyzed by RNA-seq female antennae and maxillary palps to assemble a transcriptome of 33846 contigs. Overall 79 OBPs, 88 ORs, 62 Ionotropic Receptors (IR) and 30 Gustatory Receptors (GR) were identified by comparative genomics and transcriptomics. Contigs upregulated in the antennae (620) and maxillary palps (268) were identified by differential expression (DE) and PFAM enrichments verified. We believe that a deeper knowledge of the olfactory repertoire of the tiger mosquito may help to better understand its biology and possibly it may pave the way to design new attractants/repellents.

CONTACT MAIL: fabrizio.lombardo@uniroma1.it

¹Department of Public Health and Infectious Diseases, Sapienza University of Rome, Italy

²Department of Biology, University of Naples Federico II, Naples, Italy

³Department of Life Sciences, Imperial College London, London, United Kingdom

⁴NIAID, Laboratory of Malaria and Vector Research, NIH, Maryland, USA







Genetic control of vector-borne disease by gene drive systems

Alekos Simoni

Imperial College London, United Kingdom

Invasion of alien species is posing a serious treat to human health and to ecosystems in several parts of the World. This adds to the burden of arthropods transmitted diseases which cause more than 1 million death every year. According to the World Health Organization, more than 2.5 billion people in over 100 countries are at risk of contracting dengue alone and those figures are expected to rise as a consequence of climate change and globalization. Malaria is the most deadly vector-borne disease, killing more than 430.000 people a year (WHO, 2015). The most successful control strategy of a vector-borne disease is the control of the vector that transmit it. Conventional methods include use of insecticides, bed nets, removal of breeding sites and sterile insect techniques, but in several cases those are not sufficient to eradicate a disease on a large scale. Therefore novel controls strategies are needed. We are developing genetic drive systems based on the super-Mendelian inheritance of a transgene with the aim to modify insect populations of medical or agricultural importance in a meaningful timeframe. Such modifications can for instance compromise the reproductive capacity of a vector population in order to reduce the population size to levels that do not support transmission of disease, and potentially to eradication. A potent gene drive may be able to spread despite negative fitness effects, and can therefore introduce pathogen resistance or a population suppressor which would otherwise have been rapidly removed. We are developing the technology in the malaria mosquito Anopheles gambiae, but the genetic principles and components are highly transferable to several insect pest (at least to those with a genome sequence available). Gene drive-based vector control can provide an effective, long-term, sustainable solution to the invasion and control of pest species.

CONTACT MAIL: a.simoni@imperial.ac.uk





Trento, 7-9 November 2016



Contributing to the knowledge and management of the invasive hornet *Vespa velutina*: early warning tools, sexual communication and competition with native hornets

<u>Alessandro Cini</u>¹, Gianfranco Anfora², Federico Cappa¹, Francesca Romana Dani¹, Irene Pepiciello¹, Irene Petrocelli¹, Stefano Turillazzi¹, Laura Bortolotti³, Rita Cervo¹

Slightly more than ten years ago the alien yellow legged hornet Vespa velutina (hereafter Vv) was spotted for the first time in Europe. It was immediately understood that it was a high-impact pest species, due to its huge invasion potential, habitat flexibility and specialization in honeybee predation. The Vv threat is currently faced by several European countries, but, despite the great economic interest and the large amount of researches carried out in these years, many knowledge gaps still prevent from developing efficient management approaches. Here we present the first preliminary results obtained within the framework of the national project ALTvelutina (financed by MIPAF and coordinated by CREA of Bologna) and within the STOPvelutina network. Our efforts focused on three main lines: 1) development of an early warning tool; 2) investigation on the possible existence of sex pheromone communication and 3) evaluation of the possible competition between the invasive Vv and the native hornets V. crabro (hereafter Vc). (1) The early warning method developed is based on a mobile application and a website (www.stopvelutina.it) for collecting reports from citizens and stakeholders. This early warning platform has been developed specifically for Vv, but it is easily implementable for other invasive alien species, and this meeting will represent an opportunity to attract potential interest. (2) We used an integrated approach involving behavioural, chemical and electrophysiological assays, to evaluate the possibility that Vv reproductive females release pheromones to attract males, as it has been suggested to occur in other vespine species. While we showed a male preference for reproductive females, the responsible molecules are still not identified. (3) We investigated two life history traits that might pose the invasive Vv and the native Vc in competition: i) the ability to find food sources and the flexibility in exploiting them, and ii) the immunocompetence response that might give advantage in terms of pathogen resistance. Our results show that Vv and Vc might compete for resources, and that the two species differ in their immune response. Taken together, our results set the stage for future investigations on these poorly investigated topics.

CONTACT MAIL: cini.ales@gmail.com

¹Università di Firenze-Dipartimento di Biologia, Firenze, Italy

²Centro Ricerca e Innovazione, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

³CREA - Unità di ricerca di apicoltura e bachicoltura, Bologna, Italy





Trento, 7-9 November 2016



The invasion of the Asian hornet (*Vespa velutina nigrithorax*) in France and Europe: estimating the different economic costs

Morgane Barbet-Massin¹, Jean-Michel Salles², Claire Villemant³, Quentin Rome³, Franck Courchamp¹

¹UMR 8079, Université Paris-Sud, Orsay, France

Vespa velutina nigrithorax, an Asian bee-hawking hornet, has been inadvertently introduced in southwestern France before 2004. Since its introduction, it has quickly spread through most of France and was further observed in Spain, Portugal, Italy, Belgium and Germany. Here we aimed at identifying the different types of costs (direct and indirect) arising from the spread of this invasive species. Direct costs include prevention and control such as nest destruction and trapping, research (aiming at better understanding the biology of the species and its potential impact on native species) and public education. Indirect costs mainly include a decrease of honey production and a decrease of pollination services, through the negative impact of Vespa velutina nigrithoraxon bees. Although indirect costs are difficult to estimate, the main cost coming out of Vespa velutina nigrithorax invasion is the cost of control through nest destruction (several millions of euros per year). Another important cost of Vespa velutina nigrithorax invasion is research, as several programs were founded (for over €2,000,000) to better understand the species and its potential impact on native species. Overall, the introduction and invasion of Vespa velutina nigrithorax was found to be very costly, mainly with control costs. However control through nest destruction was not proven to be useful, so public funds may be allocated differently

CONTACT MAIL: morganebm@gmail.com

²UMR LAMETA, INRA-SupAgro, Montpellier, France

³UMR 7205, Museum National d'Histoire Naturelle, Paris, France





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Novel prediction of Asian hornet invasion range and impact

<u>Alice Fournier</u>¹, Morgane Barbet-Massin¹, Quentin Rome², Claire Villemant², Colin Fontaine², Franck Courchamp¹

¹Ecologie Systématique Evolution, Univ. Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, 91400, Orsay, France

²Muséum National d'Histoire Naturelle, UMR7205, CP 50, 45 rue Buffon, 75005 Paris, France

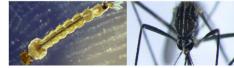
Honey bees and wild pollinators play a key role in the pollination of crops and wild plants worldwide. Their populations are currently experiencing drastic declines, due to interactions between multiple stressors: habitat loss and fragmentation, use of pesticides, climate change, pathogens and alien species. The invasion of Europe by the Asian Hornet (Vespa velutina) represents an emerging yet rapidly growing additional threat for pollinators. The hornet has already invaded a large part of France and is now spreading over the rest of Europe at an exceptionally high speed, and it is recognized as a major predator of bees. To improve the prediction of its potential future distribution, with respect to classical Species Distribution Models, I used specifically tailored predictive variables. Instead of the 19 bioclimatic variables classically used for any species, I created original expert knowledge based variables (climatic, land use and biotic inteactions), shaped according to the hornet's biological requirements. I used a combination of metrics to evaluate each variable predictive power. Since each variable type affects invasibility at a different spatial scale, I used a hierarchical procedure, applying variables successively at the scale at which they are the most influential, to obtain the final prediction. This model represents a powerful tool to identify the regions at risk of invasion by the hornet and to help managers target areas where action is needed in priority.

CONTACT MAIL: alice.fournier@u-psud.fr





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Natural and human-mediated diffusion of *Vespa velutina* in Italy and forecasting short term spread to improve management activities

Sandro Bertolino, Simone Lioy, Daniela Laurino, Aulo Manino, Marco Porporato

Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Paolo Braccini 2, 10095, Grugliasco (Turin), Italy

Implementing a cost-effective management plan for an invasive species requires the development of tools that could help to improve the performance of control activities. When the goal of the management plan is the spatial containment of a species, to understand the diffusion of the species within the present range and to evaluate where the species is most likely to spread in the short term can be really helpful to direct and localize control activities. This requires to assess the proportion of landscape that should be surveyed and the intensity of the monitoring activity allocated in each area. The yellow-legged hornet Vespa velutina is an invasive species, accidentally introduced into France in 2004 and rapidly colonizing other European countries. In Italy the species arrived in 2012, with the first nests located in 2013, and it is now spreading throughout the northwest part of the country, particularly in Liguria region. Aims of this work are to 1) reconstruct the spread of Vespa velutina since his arrival in Italy and establish a method to disentangle the natural diffusion from human-mediated transportation; 2)define buffer zones with different monitor and control intensity around the current Italian distribution range of the species with different likelihood of nests probability. The analyses were performed based on nests distribution, cluster analysis and nearest neighbour analysis of nests in respect to possible source sites of the previous year. In 2015 the frontline of the species was at 55 km along the coast from the French border, with a linear spread of 18.3 ± 3.3 km/year. A human-mediated dispersion could be recognized in different occasions. A cluster analysis of the range allowed the identification of 17 core areas used by the species, with a mean nest density of 2.9-3.5 nests/km2. Mean distances of nests observed in 2015 from possible source sites were used to define areas were the species could naturally spread in 2016. The estimated probabilities of spread were: 33.5% within 500 m from the current range, 52.8% within 1 km, 75.2% within 2 km, 92.7% within 5 km and 98.2% within 10 km. Three expansion models were then elaborated at 700, 900 and 1200 m a.s.l., considering the altitude limits were nests and adults were observed. These results could be used to improve the effectiveness of Vespa velutina control, and better focusing possible expansions areas.

CONTACT MAIL: sandro.bertolino@unito.it.





Trento, 7-9 November 2016



KEYNOTE TALK

Spread of introduced *Wolbachia* in natural insect populations: robust predictions relevant to dengue (and possibly Zika) control

Michael Turelli

Department of Evolution and Ecology, University of California, Davis, USA

Wolbachia are maternally inherited endosymbiotic bacteria that generally live inside the cells of invertebrate hosts. They can spread through populations by manipulating host reproduction to favor infected females - and they can also protect their hosts from other microbes, including viruses such as dengue and Zika that cause human disease. Since 1984, Hoffmann and Turelli have documented three examples of rapid spatial spread of Wolbachia through natural populations of Drosophila simulans, starting with spread through California at about 100 km/year. This initial example of rapid spatial spread helped motivate ongoing Wolbachia-based population manipulations aimed at dengue control in Australia, Vietnam, Indonesia, Brazil and Colombia. I discuss models and data relevant to understanding Wolbachia spread in nature. The original interpretation of Wolbachia spread invoked "bistable" frequency dynamics, in which reproductive manipulation by Wolbachia overcame both imperfect maternal transmission and fitness costs associated with the infection only after a threshold infection frequency was exceeded. However, bistability is difficult to reconcile with very rapid spatial spread. Because of new data, bistability now seems implausible for natural Wolbachia infections. Nevertheless, bistability almost certainly describes the population dynamics of artificially introduced Wolbachia infections, specifically the transinfection of Aedes aegypti with wMel, a Wolbachia strain native to D. melanogaster that suppresses the transmission of the dengue and Zika viruses. The mathematics of bistability implies that contrary to the very rapid spread of native Wolbachia through Drosophila populations, introduced Wolbachia will spread much more slowly, on the order of 100-300 m/year (versus 100 km/year), through Aedes aegypti populations. This prediction is consistent with data from population transformation trials in Australia. I discuss the robustness of analytical approximations concerning spatial spread to long-tailed dispersal and rapid local dynamics. Despite being much slower and less robust than originally expected, slow spatial spread of introduced Wolbachia through mosquito populations can greatly facilitate area-wide control of vector-borne diseases over a time scale of a few years.

CONTACT MAIL: mturelli@ucdavis.edu





KEYNOTE TALK

Are mosquito borne diseases transmitted by invasive *Aedes* mosquitoes an emerging threat for Europe?

Herve Zeller

European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Vector-borne diseases and in particular mosquito-borne diseases tend to globalise by the increasing population mobility and migration, travel and trade, which results in the fast and worldwide spread of vectors and the pathogens they transmit. Human behavioural changes (outdoor activities, habitat encroachment) have also a major impact on the exposure to vectors. Mosquito-borne diseases liaised to invasive species e.g. Aedes aegypti or Ae. albopictus represent a threat in Europe with their widespread establishment in the recent decades in several European and neighbouring countries. The expanded transmission of Aedestransmitted diseases such as dengue, Chikungunya, Yellow fever and Zika in tropical and sub-tropical countries and the growing number of potentially exposed travellers returning from these areas increases the risk of upsurge of local outbreaks in infested regions in Europe. Already several alerts have been reported in Europe in the recent years. All these emergences were linked to introduction of infectious traveller returning from an affected area with sub-sequent local transmission by mosquitoes. Countries and regions infested by invasive mosquito vectors should analyse carefully the lessons learnt from these different European upsurges, as well as in other areas in the world facing similar challenges. They should anticipate these threats and develop preparedness plans. Surveillance of invasive mosquitoes in Europe should be strengthened with close monitoring to better define the suitable periods for potential transmission. Limited tools are available to control these invasive mosquitoes and resistance to insecticides should be closely monitored to better define cost-effective strategies for vector control. Innovative approaches should be developed to control these mosquito populations both in tropical/sub-tropical and temperate regions with particular focus on urbanized areas. The changing epidemiology of invasive mosquito-borne diseases in Europe challenges the public health authorities. Collaboration between multiple disciplines, responsible human, and environmental authorities will be pivotal for early detection, effective surveillance, adequate risk assessment, communication, response and control of these mosquito-borne diseases.

CONTACT MAIL: herve.zeller@ecdc.europa.eu





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Global invasion of *Aedes albopictus* as a vector of arbovirus: what consequences for disease outbreaks?

<u>Giuliano Gasperi</u>¹, Mosè Manni¹, Carmela R. Guglielmino¹, Michele Marconcini¹, Francesca Scolari¹, Anubis Vega-Rua², Anna-Bella Failloux³, Anna R. Malacrida¹

Invasive species represent a global concern for their rapid spread and the possibility of infectious disease transmission. This is the case of the global invader Aedes albopictus, the Asian tiger mosquito, vector of more than 20 arbovirus, notably chikungunya (CHIKV), dengue (DENV), Zika (ZIKV). The identification of important invasion corridors has great potential for mitigating invasion and disease risks. Combining classical population genetics and Approximate Bayesian Computation (ABC) approaches with historical records we provide a comprehensive picture of the demographic history of populations from the supposed native range, South East Asia, and from the invaded regions in Indian Ocean, Mediterranean Basin and North and South America. In South East Asia, the low differentiation and the high co-ancestry values identified among China, Thailand and Japan confirm that, in the native range, these populations maintain high genetic connectivity, revealing their ancestral common origin. China appears as the oldest one. Outside Asia, the invasion process in Reunion, Americas and the Mediterranean Basin is supported by a chaotic propagule distribution, which co-operate in maintaining a relatively high genetic diversity within the adventive populations. We propose that the demographic history of the populations may contribute to create the conditions for efficient arbovirus transmission and, consequently, for outbreak establishment.

CONTACT MAIL: gasperi@unipv.it

¹Department of Biology and Biotechnology, University of Pavia, 27100 Pavia, Italy

²Laboratory of Medical Entomology, Environment and Health, Institut Pasteur of Guadeloupe, 97183 Abymes, Guadeloupe, France

³Department of Virology, Laboratory of Arboviruses and Insect Vectors, Institut Pasteur, 75724 Paris, France





FACING THE INVASION OF ALIEN ARTHROPODS SPECIES

ecology, modelling and control of their economic impact and public health implication Trento, 7-9 November 2016



Cold-hardiness of the Asian tiger mosquito *Aedes albopictus*, a vector of multiple viral and parasitic pathogens

Ruth Müller¹, Aljoscha Kreß², Ann-Marie Oppold^{2,3}, Ulrich Kuch¹

¹Goethe University Frankfurt am Main, Institute of Occupational Medicine, Social Medicine and Environmental Medicine, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany

²Senckenberg Biodiversity and Climate Research Centre, Department Climate and Adaptation, Senckenberganlage 25, 60325 Frankfurt am Main, Germany

³Goethe University Frankfurt am Main, Molecular Ecology Group, Institute for Ecology, Evolution & Diversity, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany

Background and objectives: The Asian tiger mosquito *Aedes albopictus* is an important threat to public health due to its rapid spread and its well-recognized role as a vector for dengue and other viruses as well as zoonotic parasites. The rapid development of low-temperature phenotypes after introduction to temperate zones has been identified as a key trait for its successful establishment. The aim of present study is to elucidate the species overwintering success in temperate, relatively cool ecoregions.

Methods and Methods: The mosquito eggs produced by adults from different photoregimes (winter versus summer) were exposed to 3°C and used for a detailed morphometric analysis at the ultra-structural level. In addition, mosquito eggs were stepwise cooled down to -2°C, exposed for different times and, after stepwise warming, the larval hatching success from the eggs was determined.

Results: Our detailed metric analysis of *Ae. albopictus* eggs reveals different ultrastructural alterations produced by photoperiod (decreased thickness of the dark endochorion) and cold acclimatization (increased thickness of the middle serosal cuticle). Temperatures ≥0°C do not change the larval hatching success from eggs. An exposure to -2°C reduces the larval hatching success to 50% after 9.5 to 63.3 hours.

Conclusion: The gained knowledge improves the estimation of the vector's overwintering success in temperate regions and thereby the risk assessment of *Ae. albopictus* associated diseases in Europe.

Keywords: mosquito-borne diseases, overwintering, vector biology

CONTACT MAIL: Ruth.Mueller@med.uni-frankfurt.de





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Climate change and expansion of mosquito-borne viral diseases in the Hindu Kush Himalayan region

Meghnath Dhimal^{1,2,3}, Ulrich Kuch², David A. Groneberg², Bodo Ahrens³, Ruth Müller ²

¹Nepal Health Research Council (NHRC), Ministry of Health Complex, Kathmandu, Nepal

Although hydro-meteorological data for the Hindu-Kush Himalayan (HKH) region are scarce, the available data indicate that its warming trend is more pronounced than global trend. Accordingly, a shift of disease vectors and disease transmission to higher elevations is predicted under both observed and future climate change scenarios. At least four major mosquito-borne viral diseases (MBVDs) namely, Japanese encephalitis, West Nile virus, chikungunya and dengue fever are endemic in HKH region, all caused by pathogens that are transmitted to or among humans by mosquitoes that are sensitive to temperature and thus, ultimately, to climate change. For this study, we systematically reviewed and summarized information on climate change and the distribution of VBVDs in HKH region from the published literature until July 2016 including our own research studies of vector-borne diseases in Nepal. We found a distinct shift of MBVD incidences with an expansion of autochthonous cases to previously non-endemic areas including mountain regions. The distribution of the diseases and their vectors, which were previously believed to be confined to the tropical and sub-tropical region, is now observed to extend to the hills and mountains of HKH region. Given the establishment of relevant disease vectors at altitudes of already at least 2,000 m above sea level, increasing movement of people between endemic and non-endemic areas, urbanization and poverty, climate change can intensify the risk of MBVD epidemics in the fragile HKH region. Finally, the wider distribution of these important disease vectors in regions that had previously been considered to be non-endemic calls for a regional collaboration for extending and scaling-up MBVD surveillance and control programs in HKH region to protect the health of both local people and mountain tourists.

CONTACT MAIL: meghdhimal@nhrc.org.np

²Institute of Occupational Medicine, Social Medicine and Environmental Medicine, Goethe University, Frankfurt am Main, Germany

³Institute for Atmospheric and Environmental Sciences (IAU), Goethe University, Frankfurt am Main, Germany





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Codon usage and phylogenetic analyses elucidate the recent evolutionary history of Zika virus

Lino Ometto, Omar Rota-Stabelli

Unit of Agricultural Entomology, Department of Sustainable Agro-ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

As all viruses, Zika relies on the host replication machinery and is under selection to use codons matching the optimal usage of the host. Here, we analyzed the adaptation of Zika to the codon usage of its primary vectors, *Homo sapiens* and *Aedes aegypti*, as well of *Pan troglodytes* (chimpanzee), *Aedes albopictus*, and *Culex* (*pipiens*) *quinquefaciatus*. Our results indicate strong codon adaptation of Zika to *Ae. aegypti*, humans and chimpanzees, confirming a long-term association with these hosts. On the other hand, the poor adaptation to the codon usage of *Ae. albopictus* suggests a relatively inefficient vector capability of this species. We found clear indication that codon usage has changed in the Asian and American lineages of Zika, which may explain their high virulence and related recent outbreaks. Indeed, phylogenetic and clock analyses revealed differentiation of the American lineages, which may indicate fast evolution driven by adaptation. We also confirm a single introduction in South America earlier in time than previously proposed, and show that this introduction was followed by a fast radiation of at least 6 lineages. Our evolutionary analyses clarify the epidemiology of Zika and indicate that codon adaptation can have a diagnostic value for host and vector suitability.

Contact mail: lino.ometto@gmail.com







Insights into the bacterial microbiota components of vectors circulating in Marche region, Italy

Maria Vittoria Mancini, Guido Favia, Claudia Damiani

Scuola di Bioscienze e Medicina Veterinaria, University of Camerino, Camerino, Macerata, Italy

Mosquito-borne diseases represent worldwide anthroponotic and zoonotic medical burdens: pathogenic agents, including protozoan parasites (malaria), viruses (Dengue, West Nile, Chikungunya and Zika) together with filariae, pose relevant broad concerns in public health and economics. Alongside representing the most severe causes of human mortality and morbidity throughout the Tropics, some of these diseases are subjected to re-emergence phenomena, creating concerns in whole Europe. In Italy, the major alarms for humans arise from the highly invading and aggressive Aedes albopictus and from mosquitoes of the Culex and Culiseta genera, vectors of numerous and diverse arboviruses, whose outbreaks (West Nile and Chikungunya) are becoming increasingly frequent in North and Centre-Italy. Moreover, many etiological agents of zoonosis, are also transmitted by vectors: for example, the Bluetongue virus (BTV), transmitted by biting midges (Culicoides sp.) is becoming an increasingly important threat among ruminants, causing significant financial losses in affected farms. The complexity of these vectorial systems will require the establishment of interdisciplinary measures and multi-targeting approaches able to investigate physiology, ecology and behavioural changes of vectors. In this frame, a crucial player is represented by mosquito microbiota. The consortia of microbiota inhabiting metazoa are now recognized as pivotal driving force contributing to various aspects of their hosts, including overall physiology, nutrient metabolism and immune system activation, but also feeding behaviour, reproduction, community interactions and, interestingly, vector competence.

Here we present a large spectrum microbial profile of diverse mosquito's genera and species circulating in Marche Region, particularly in the area of San Benedetto del Tronto (AP): field-collected *Aedes albopictus*, *Culex pipiens* and *Culiseta longiareolata* were microbiologically and molecularly analysed and their microbiota components disclosed. Additionally, *Culicoides*, belonging to the obsoletus complex, were included in this survey: for the first time, microbiota components of BTV vectors were evaluated by Next Generation Sequencing approaches. Understanding the increasingly apparent deep synergism between resident microbiota and hosts in numerous vectors of diseases, not only provides crucial insights on their biology, but it also opens real and applicable possibilities for symbiont-based approaches for their control.

CONTACT MAIL: claudia.damiani@unicam.it and mariavittoria.mancini@unicam.it





FACING THE INVASION OF ALIEN ARTHROPODS SPECIES

ecology, modelling and control of their economic impact and public health implication **Trento, 7-9 November 2016**



Surveillance of invasive mosquitoes in Switzerland

<u>Laura Vavassori</u>^{1,2}, Pie Müller^{1,2,3}, Lukas Engeler³, Tobias Suter^{1,2,4}, Eleonora Flacio³, Valeria Guidi³, Mauro Tonolla³

¹Vector Control Group, Epidemiology and Public Health Department, Swiss Tropical and Public Health Institute, Socinstrasse 57, PO Box, CH-4002 Basel, Switzerland

Due to the continued spread of *A. albopictus* observed in the Canton of Ticino over the last decade, a systematic surveillance at the country scale has been initiated in 2013. The surveillance consists of a network with ovi- and BG-sentinel traps placed at 38 potential points of entry, including motorway services stations, ports and airports. Each year, the traps have been systematically monitored biweekly from June to September. Mosquito specimens collected are identified to species level by morphology and matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Over the last 3years, alongside several indigenous mosquitoes, also 3 invasive *Aedes* species have been recorded across Switzerland, including *A. albopictus*, *A. japonicus* and *A. koreicus*. In this paper, we will present the observed patterns in the spatial and temporal distribution of these invasive mosquitoes, and discuss the findings in the context of mosquito and disease control at the national and European level.

CONTACT MAIL: l.vavassori@unibas.ch

²University of Basel, Petersplatz 1, CH-4003 Basel, Switzerland

³Applied Microbiology Lab, University of Applied Sciences and Arts of Southern Switzerland, Via Mirasole 22a, CH-6501 Bellinzona, Switzerland

⁴Avia-GIS, Risschotlei 33, 2980 Zoersel, Belgium







Exotic mosquito surveys in Piedmont

<u>Andrea Mosca</u>¹, Matteo Giovannozzi¹, Mirko Perna¹, Cristina Grieco¹, Mario Di Giulio², Paolo Roberto¹

¹Piedmont Mosquito Abatement District - IPLA S.p.A., Corso Casale 476, I-10124 Torino, Italy ²USMAF Torino Caselle, Italy

Piedmont, the largest of the north-western regions of Italy, is for two-thirds surrounded by mountains, but it is also crossed by important roads and international trades which do not put it away from possible introduction of invasive mosquitoes. Indeed, the Asian tiger mosquito was accidentally introduced in the 90s of the last century. Due to the presence of other invasive species in surrounding regions (e.g. Ae. japonicus in Canton of Ticino, Ae. koreicus in Lombardy) and on how easily such these species have been transported around the world, Ipla, with the regional government mandate, has set up a mosquito surveillance plan for the early detection of the introduction of exotic/invasive mosquitoes in about 60 known high risk entry points: international airports, rolling highway terminals, container terminals, customs areas, companies importing flowers and used tires from abroad. Each point has been ranked according to risk-based criteria resulting from a questionnaire and an objective analysis of the location. Criteria include geographical characteristics, quantity of imported goods, their origin, presence and abundance of larval breeding sites etc. Larval inspections, adult samplings and ovoposition traps were employed, individually or in different combinations, depending on the potential rank of each site. Furthermore, to investigate the presence of mosquitoes on flights coming from counties with overt presence of exotic mosquitoes or/and mosquito born disease to Turin airport, passenger cabins of these flights are inspected for the presence of live mosquitoes upon arrival. Results of the 2016 surveillance plan in Piedmont will be presented.

CONTACT MAIL: zanzare@ipla.org





FACING THE INVASION OF ALIEN ARTHROPODS SPECIES

ecology, modelling and control of their economic impact and public health implication **Trento, 7-9 November 2016**



Invasive mosquitoes keep spreading in Northern Italy: first report of *Aedes koreicus in* the Liguria region

Marco Ballardini¹, Alessandra Pautasso², Giorgio Chiaranz³, Stefano Ferretti⁴, Maria Vittoria Riina², Maria Cristina Radaelli², Federica Verna², Umberto Cardellino⁵, Ilaria Cremonesi⁶, Massimo Serra⁶, Marina De Camilli⁷, Serena Durante¹, Annalisa Accorsi¹, Pier Luigi Acutis², Cristina Casalone², Walter Mignone¹

Entomological and virological surveillance on mosquitoes in North-western Italy (Piedmont, Liguria and Valle d'Aosta Regions) is being conducted by IZSPLV since 2011, in the framework of research projects funded by the Ministry of Health and the European Union. In 2016, mosquito surveillance in the Liguria Region was intensified, thanks to the support of the Regional administration and the collaboration of the public Veterinary Services. The invasive alien mosquito Aedes albopictus, an important vector of viral diseases, is the most abundant species in the area, where it was first found in the city of Genova in 1990. From mid June to late October, as many as 23 sites are being monitored fortnightly by means of mosquito traps, as well as standard ovitraps. Adult mosquitoes are identified to the species level based on morphological keys, and eggs are reared in laboratory to obtain adult specimens. Particular attention is being paid to the city of Genova (10 sites out of 23), due to the presence of several sites at risk of importing invasive alien mosquito species. Between 29 June and 19 July 2016, 3 adult female Aedes koreicus specimens were caught in 2 sites located in Genova, by means of a Gravid trap (N=2) and a BG-sentinel trap baited with CO2 and a lure (N=1). The 2 sites are 1.1 Km apart and one of them is located in front of the port commercial area. The presence of a clear basal pale band on the hind tarsomere V of all specimens is consistent with the phenotype already detected in Belgium and North-Eastern Italy. Bio-molecular assays to confirm the species identity, based on COI and ND4 mitochondrial markers, are currently in progress. Ae. koreicus is an Asiatic mosquito which has become invasive in Europe in the recent years. Monitoring in Genova is being intensified further to clarify whether this alien species has established in the area, and locate possible breeding sites. While the origin of these specimens remains unknown, the presence of Ae. koreicus in such an important commercial and tourism hub is worrisome, as it might strongly accelerate the species' spread in Italy and in the rest of Europe, as already happened with Ae. albopictus.

CONTACT MAIL: marco.ballardini@izsto.it

¹Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSPLV), Imperia, Italy

²Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSPLV), Torino, Italy

³Il Rastrello Cooperativa Sociale a r.l. - Onlus, Genova, Italy

⁴Centro Studi BioNaturalistici S.r.l., Genova, Italy

⁵S.C. Sanità Animale, ASL 3 Genovese, Genova, Italy

⁶Dipartimento Salute e Servizi Sociali, Regione Liguria, Genova, Italy

⁷Ufficio Animali, Comune di Genova, Genova, Italy





Trento, 7-9 November 2016



Development of novel non-conventional approaches to monitor *Aedes albopictus* abundance and biting rates

<u>Beniamino Caputo</u>¹, Mattia Manica¹, Pietro Cobre¹, Federico Filipponi¹, Cesare Bianchi², Alessandra della Torre¹

¹Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma Sapienza, Piazzale Aldo Moro 5, 00185 Rome, Italy

²Dipartimento di Fisica, Università di Roma Sapienza, Piazzale Aldo Moro 5, 00185 Rome, Italy

Estimates of Aedes albopictus abundance and biting rates are essential to account for vectorhuman contact and to predict nuisance threshold and risk of arbovirus autochthonous transmission useful for correct planning of mosquito control interventions. Novel approaches to monitor this daytime and outdoor biting mosquito and assess its abundance and biting rates are deeply needed, as current methods have low effectiveness and/or are expensive and/or difficult to be implemented in large scale. In 2016 Aedes albopictus reproductive season, we focused on the development of two non-conventional approaches to monitor Ae. albopictus abundance and biting rates. The first approach is represented by a mobile application named ZANZAMAPP which allows users to easily record geo-referenced reports of spotted mosquitoes including basic information on time, indoor/outdoor location and number of bites. Analysis of the provided records (>10.000 in the first 2 months) has the potential to provide a dynamic spatial map of mosquito abundance and human perception of mosquito nuisance in Italy. Once optimized and validated it could represent a cheap and effective way for public administration to gather information relevant for the planning of mosquito control, provided that they engage themselves in the promotion of ZANZAMAPP use by their citizens. The second approach is a citizen science pilot project in which we involved students of the Faculty of Medicine of Sapienza University in the monitoring of Ae. albopictus, by providing them with a sticky-trap patented by our research group and asking them to: i) report number, species and gender of weekly collected specimens; ii) send us by mobile phone photos of the sticky sheets for double-check of specimens identifications; and iii) fill a simple questionnaire on their perception of mosquito nuisance in the week preceding each collection. Analysis of the provided information will allow to assess whether non-experienced people with a short training can effectively contribute to a capillary monitoring of the species and provide the basis for a larger citizen science project in future years. The talk will focus on the description and preliminary results of both these two novel approaches and highlight their potential to complement entomological surveillance of Ae. albopictus.

CONTACT MAIL: beniamino.caputo@uniroma1.it





Trento, 7-9 November 2016



Estimating *Aedes albopictus* biting females by ovitrap surveillance data in an area at high-risk of exotic arbovirus introduction

<u>Mattia Manica</u>¹, Antonello D'Alessandro¹, Roberto Rosà², Alessandra della Torre¹, Beniamino Caputo¹

¹Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma "Sapienza", Piazzale Aldo Moro 5, 00185 Rome, Italy

²Dipartimento di Biodiversità ed Ecologia Molecolare, Centro Ricerca e Innovazione, Fondazione Edmund Mach, 38010 San Michele all'Adige, TN, Italy

Aedes albopictus is an aggressive invasive mosquito species which represents a serious health concerns in both tropical and temperate regions due to its role as vector of arboviruses. Estimates of mosquito biting rates are essential to account for vector-human contact in models aimed to predict the risk of arbovirus autochthonous transmission and outbreaks as well as nuisance threshold useful for planning mosquito control interventions. Methods targeting daytime and outdoor biting Ae. albopictus females (e.g. Human Landing Collection, HLC) are expensive and difficult to be implemented in large scale schemes. Instead, egg-collections by ovitraps is the most widely used routine approach for large-scale monitoring of the species. We here present the first attempt to provide: i) rough estimates of adult biting Ae. albopictus females based on ovitrap data, ii) straightforward indicators to be exploited by decision-makers in charge of planning mosquito-control activities. We carried out parallel ovitrap and human landing collections in hotspots of high Ae. albopictus abundance in Rome (Italy) along a whole reproductive season and assessed the relationship between the two sets of data by regression analysis. The mean number of females/person collected by HLC in 15' (hereafter females/HLC) and the mean number of eggs/day were 18.9±0.7 and 39.0±2.0, respectively. Results shown a significant positive relationship between the two sets of data and estimated an increase of one biting female/person/day every 5 additional eggs found in ovitraps. Both observed and fitted values indicated presence of adults in the absence eggs in ovitraps. The patterns of exotic arbovirus outbreak probability obtained by introducing these estimates in risk models were in the range of those based on females/HLC (RO>1 in 86% and 40% of sampling dates for Chikungunya and Zika, respectively; R0<1 along the entire season for Dengue). However, the model predicted that under our experimental conditions an R0>1 for Chikungunya is to be expected at very low number of eggs/ovitrap and even in their absence. Overall, large confidence intervals in the model predictions warn about the significance of relying on ovitrap monitoring schemes to estimate numbers of biting females and plan control interventions aimed to prevent risk of arbovirus transmission or of high nuisance.

CONTACT MAIL: <u>mattia.manica@uniroma1.it</u>





KEYNOTE TALK

Targeting Aedes albopictus in a metropolitan European area

Alessandra della Torre, Mattia Manica, Beniamino Caputo

Department of Public Health & Infectious Diseases, University of Rome Sapienza, Rome, Italy

Accuracy of mathematical and statistical models to assess the risk of autochthonous transmission of exotic arboviruses such as Dengue, Chikungunya and Zika in Europe relies on appropriate estimates of biological parameters related to the potential mosquito vector population. However, information on the biology of invasive *Aedes albopictus* in southern European urban areas is scarce and these biological parameters are usually derived from data available from other continents or from laboratory experiments. Since the establishment of a very stable and abundant *Ae. albopictus* in Rome almost 20 years ago, our group has focused on studying epidemiologically relevant behaviours of the species within the metropolitan area, as well as in neighbouring areas, taking advantage of novel tools for collection an monitoring of adult mosquito samples. The talk will particularly focus on the following parameters: i) seasonal dynamics; ii) spatial distribution and its eco-climatic determinants; iii) dispersal; iv) feeding habits and v) human-mosquito contact. In addition to present an overview of the data collected in the last years, the talk will also focus on the assessment of the effectiveness of vector control intervention (i.e. larvicide treatments of rain catch basins associated to period adulticide spraying) routinely carried out in Italian urban areas to reduce the nuisance caused by this very aggressive daytime biting species.

CONTACT MAIL: alessandra.dellatorre@uniroma1.it





ecology, modelling and control of their economic impact and public health implication Trento, 7-9 November 2016



Insecticides and essential oils against the Asian bush mosquito

<u>Friederike Reuss</u>^{1,2}, Johannes Wilhelm³, Dennis Knauß⁴, Ulrich Kuch⁴, Markus Pfenninger^{1,2}, Ruth Müller⁴

¹Institute for Ecology, Evolution and Diversity, Faculty of Biological Science, Department of Molecular Ecology, Goethe University, Max-von-Laue-Straße 9, 60438 Frankfurt am Main, Germany

²Senckenberg Biodiversity and Climate Research Centre, Molecular Ecology, Senckenberganlage 25, 60325 Frankfurt am Main, Germany

³Institute for Ecology, Evolution and Diversity, Faculty of Biological Science, Department of Aquatic Ecotoxicology, Goethe University, Max-von-Laue-Straße 9, 60438 Frankfurt am Main, Germany ⁴Institute of Occupational Medicine, Social Medicine and Environmental Medicine, Department of Environmental Toxicology and Medical Entomology, Goethe University, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany

The Asian bush mosquito (*Aedes japonicus japonicus*; Diptera: Culicidae) is an invasive species which became established in Europe during the last decades. Its rapid spread within Germany and its potential to be the dominant species among container mosquitoes may force us to combat it in the future to prevent nuisance and pathogen transmission. We therefore tested the effect of insecticides and essential oils on the oviposition in the field and on the larval performance of this species in lab experiments. We show that clove and lavender oil reduce the number of oviposited eggs. In addition, we showed that the common insecticides Bacillus thuringiensis israelensis and pyrethrum extract and clove oil have a larvicidal effect. Temperature has been shown to alter larval toxicity of insecticides. Essential oils may be an alternative to known insecticides to control the Asian bush mosquito.

CONTACT MAIL: friederike.reuss@senckenberg.de





FACING THE INVASION OF ALIEN ARTHROPODS SPECIES

ecology, modelling and control of their economic impact and public health implication **Trento, 7-9 November 2016**



Resistance to pyrethroids in *Aedes albopictus*: results from a multi-country survey

Mariangela Bonizzoni¹, Jiabao Xu², Xiaoguang Chen², Guiyun Yan³

The Asian tiger mosquito Aedes albopictusis an invasive mosquito with public health relevance because it is a competent vector for a number of viruses, including Dengue, Zika, Usutu and Chikungunya and the nematode *Dirofilaria immitis*. The presence of *Ae. albopictus* in Europe has already been linked to sporadic cases of autochthonous transmission of Dengue in France and a small epidemic of Chikungunya in Italy. Aedes albopictus arrived in Europe at the end of the '1970 from Asia and, because it has a high ecological plasticity, it was able to reach as far north as the Netherlands, even if it is mostly established in countries facing the Mediterranean Sea. Currently, there are neither licensed vaccines nor specific therapeutic treatments for viruses vector by Ae. albopictus, leaving control of vector populations the only available tool to prevent disease transmission. Pyrethroids are widely used to control adult mosquitoes, imposing selection pressure for the emergence of resistance. We surveyed mutations in the pyrethroid target site, the para sodium channel gene, of 597 Ae. albopictus specimens across Asia, Africa, America and Europe, including two samples from Italy. Additionally, we established an association between mutations at the para sodium channel gene and the resistance phenotype by genotyping two populations from China, which had been previously phenotyped using the World Health Organization standard tube bioassay for pyrehtroid resistance. We detected two non-synonymous mutations in 5 populations across 4 countries. Most prevalent mutations were at position 1534, where we detected changes from phenylalanine to cysteine or serine or leucine. Positive association between pyrehtroid resistance and the presence of the F1532S mutation was established in two Chinese populations. Our results suggest that resistance to pyretrhoids is emerging in Ae. albopictus and should be carefully monitored to favor the sustainable use of this type of insecticides. Mutations in the para sodium channel gene can be used as biomarkers for resistance surveillance, but not all mutations have the same predictive power.

CONTACT MAIL: m.bonizzoni@unipv.it

¹Biology and Biotechnology Department, University of Pavia, Pavia, Italy

²Parasitology Department, Southern Medical University, Guangzhou, China

³Public Health, University of California at Irvine, Irvine, CA, USA





ecology, modelling and control of their economic impact and public health implication **Trento, 7-9 November 2016**



Susceptibility of Italian *Aedes albopictus* and *Culex pipiens* populations to insecticides most widely used in interventions against adult mosquitoes

<u>Verena Pichler</u>¹, Mattia Manica¹, Pietro Cobre¹, Joao Pinto², Alessandra della Torre¹, Beniamino Caputo¹

¹Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma La Sapienza, Roma, Italy ²Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universida de Nova de Lisboa, Lisbona, Portugal

The invasive Aedes albopictus and the indigenous Culex pipiens are the most widely spread mosquito species in urban areas in Italy. Both species not only create considerable nuisance to citizens, but are also vectors of arboviruses such as Chikungunya, Dengue, Zika (Ae. albopictus) and West-Nile (Cx. pipiens). Although insecticide-based interventions are carried out by citizens and public administrations in order to reduce the nuisance, little is known on the susceptibility of these species to insecticides. We carried out a first assessment of levels of resistance of field populations of both species sampled along the Italian peninsula to the most commonly used pyrethroid insecticides in Italy (i.e. Permethrin, Deltamethrin and Cypermethrin). Resistance tests were performed following WHO protocols and mortality was recorded24 hours after a 1-h-exposure. Three to 4 replicates were carried out for each population. Preliminary results obtained for Ae. albopictus and Cx. pipiens populations collected in 2015 in two sites in Rome highlighted first signs of reduced susceptibility to Permethrin in Ae. albopictus (96% mortality) never reported before in Italian populations, and higher resistance in Cx. pipiens populations (73.3% mortality) collected in the same larval breeding site. To extend this preliminary study, we requested Ae. albopictus and Cx. pipiens eggs and/or larval samples to colleagues from Institution in various Italian regions, as follows: Trentino; Veneto; Piemonte; Liguria; Emilia Romagna; Marche; Lazio; Abbruzzo; Bari; Messina. Insecticide resistance tests will be performed as above. Results will represent the first assessment of susceptibility of the two most common urban mosquito species in Italy and constitute a fundamental baseline data to monitor raising of resistance in the next years. In particular, results will be discussed with reference to: - susceptibility to insecticides in the two species across Italy; - susceptibility to insecticides in areas with vs. without insecticide treatments; - susceptibility to insecticides in sympatric populations of the two species.

CONTACT MAIL: verena.pichler@uniroma1.it







KEYNOTE TALK

Population dynamics of endemic mosquitoes species and epidemiological implications in Northern Italy

Stefano Merler

Fondazione Bruno Kessler, Trento, Italy

The rapid invasion and spread of Aedes albopictus within new continents and climatic ranges has created favorable conditions for the emergence of tropical arboviral diseases in the invaded areas. We used mosquito abundance data from 2014 and 2015 collected across ten sites in northern Italy to calibrate a population model for Ae. albopictus and to estimate the transmission potential of chikungunya, dengue and ZIKV. The model predicts a significant risk of chikungunya outbreaks in most sites if a case is imported between the beginning of summer and up to mid-November, with an average outbreak probability between 4.9% and 25%. Peak values of R0 range from 0.5 to 3 depending on the site. A lower risk is predicted for dengue, with an average probability between 4.2% and 10.8% for cases imported between mid-July and mid-September and peak values of RO from 0.5 to 2. Importantly, R0 of dengue remains above the epidemic threshold for a much shorter period of time. We estimated the basic reproductive number R0for ZIKV to be systematically below the epidemic threshold in most scenarios. The main determinants of transmissibility are i) local abundance of Ae. albopictus, which in turn depends on local temperature patterns, precipitation - a robust negative correlation was found with late spring precipitations - and habitat suitability, and ii) natural history of the diseases. In particular, the lower estimated risk for dengue is mainly determined by the length of the extrinsic incubation period in Ae. albopictus, while the lower risk of ZIKV is mainly ascribable to the low transmission efficiency of Ae. albopictus, compared for instance to that of Ae. aegypti.

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CONTACT MAIL: merler@fbk.eu





Trento, 7-9 November 2016



Determination of ecological parameters of the invasive mosquito species *Aedes* albopictus and *Aedes koreicus* in a mountainous environment of Northeastern Italy and experimental and applied control

<u>Gioia Capelli</u>¹, Frederic Alexandre Baldacchino², Daniele Arnoldi², Silvia Ravagnan¹, Francesca Bussola², Roberto Rosà², Graziana Da Rold¹, Sara Carlin¹, Andrea Drago³, Fabrizio Montarsi¹, Annapaola Rizzoli²

¹Laboratory of Parasitology, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy ²Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy ³Entostudio, Ponte San Nicolò (PD), Italy

The Pre-Alps and Alps of Northeastern Italy (Veneto region and Trento province) are currently colonized by the established Invasive Mosquito Species (IMS) Aedes albopictus and by the newly arrived Aedes koreicus. Their introduction and spread pose new threats to human and animalhealth, due to their known or potential vector competence of endemic and exotic pathogens, and complicate the entomological surveillance. Modelling is a powerful tool to explain and predict the possible invasion and expansion of IMS in new territories, provided that reliable ecological parameters are available from laboratory and field studies. Here we report the results of a three-year survey as well as laboratory experiments on the two IMS present in the area Ae. albopictus and Ae. koreicus, aimed to gather information on their spatio-temporal distribution in Northeastern Italy, habitat characterization, biting behavior and the effect of temperature on the life cycle. All these data were then used to model the potential distribution of IMS in Italy under different climate change scenarios. In addition, the potential risk of arboviruses epidemics was estimated in temperate areas with established populations of Ae. albopictus. Other aspects were explored to improve the monitoring of IMS, such as the performance of three trapping devices and new methodologies of adults and eggs identification based on mass spectrometry. Finally, new methodologies of mosquito control were evaluated, i.e. predation tests of copepods on Aedes larvae and an integrated control strategy with a community-based approach including larvicide applications in public and private areas combined with a door-to-door active education to involve residents in mosquito control.

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CONTACT MAIL: gcapelli@izsvenezie.it





Trento, 7-9 November 2016



Potential distribution of invasive species in Italy

<u>Markus Metz¹</u>, Frederic Alexandre Baldacchino¹, Gioia Capelli², Daniele Arnoldi¹, Matteo Marcantonio¹, Fabrizio Montarsi², Markus Neteler³, Annapaola Rizzoli¹

¹Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

²Laboratory of Parasitology, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy ³Mundialis GmbH & Co. KG, Bonn, Germany

The invasive species Aedes albopictus, Aedes koreicus have been invading not only Italy but also other countries in Europe in the last years. While Ae. albopictus is by now well established in Italy, Ae. koreicus is still spreading. The Asian tiger mosquito Ae. albopictus is not only a biting nuisance to humans, it can also act as a vector for various arboviruses, e.g. Dengue, Chikungunya and Zika. The potential of Ae. koreicus to transmit disease agents is yet unclear, but it is also a biting nuisance and even more anthropophilic than Ae. albopictus. Here we developed datasets describing the current climatic conditions in Europe, representative for the years 2003 – 2012. The main datasets were reconstructed MODIS land surface temperature time series data and time series of precipitation estimates from satellite observations calibrated against meteorological station data. These datasets in turn were used to model the potential distribution of the focus species. Climatic parameters relevant for each species were obtained from literature and specific thresholds were determined from current trap data collected within the LExEM project. Highresolution (250 m) potential distribution maps were obtained from the respective climatic parameters with their specific thresholds and the climatic datasets. Further on, we used climate model predictions from CMIP5 to estimate future changes in the potential distribution for Ae. albopictus and Ae. koreicus for the years 2030 and 2050.

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CONTACT MAIL: markus.metz@fu-berlin.de





Trento, 7-9 November 2016



Preventing the risk of Chikungunya outbreaks through vector control programs in temperate climate countries: a cost-utility analysis in the epidemic scenario of Emilia Romagna

Filippo Trentini¹, Piero Poletti^{1,2}, Alessia Melegaro¹

¹Dondena Center – Bocconi University, Italy

In 2007, Italy has experienced the first large epidemic outbreak documented in a temperate climate country caused by Chikungunya virus, with approximately 161 laboratory confirmed cases concentrated in two bordering villages in the Emilia Romagna region. Although Chikungunya is not yet an endemic disease in Europe, as a consequence of globalization, the risk of observing future outbreaks of tropical vector-borne diseases is increasing in temperate climate countries. It becomes therefore critical to assess, both from an epidemiological and an economic point of view, which are the most effective and cost-effective control measures for controlling potential vectorborne epidemics. A previously published mathematical model [Poletti et al. (2011) "Transmission Potential of Chikungunya Virus and Control Measures: The Case of Italy", PLoS ONE] is here used to describe the temporal dynamics of the competent vector, known as Aedes albopictus, taking into account climatic factors and the infection transmission mechanism driving the spread of the epidemic in both mosquitoes and humans. The model is calibrated on the observed epidemiological data of the Emilia Romagna 2007 epidemic and further extended to simulate a variety of different epidemiological scenarios and intervention strategies based on breeding sites removal, larvicidal and adulticidal programs. A cost-utility analysis with a stochastic approach to account for uncertainty on incidence of cases provided by model predictions, on costs and on DALYs loss was performed. The effects of uncertainty around the arrival of the primary case affected with Chikungunya and the time to intervention on the effectiveness and costeffectiveness of different control measures is also explored. Model results show the most costeffective and cost-saving interventions in reducing the burden of Chikungunya disease in different plausible epidemiological situations. Although the transmission potential of Chikungunya virus is sensibly high, our results strongly suggest that possibile epidemics can be controlled by performing a timely and cost-effective strategy. Optimal interventions are highlighted in relation to when the potential outbreak occurs during the mosquito season. The framework developed can be extended to other regions or countries to inform the implementation of optimal preventive measures.

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CONTACT MAIL: trentini.filippo@unibocconi.it

²Fondazione Bruno Kessler, Italy





Trento, 7-9 November 2016



KEYNOTE TALK

Current status of the *Drosophila suzukii* management in Trentino (Italy), research achievements and perspectives for sustainable control

<u>Claudio Ioriatti</u>¹, Gianfranco Anfora², Alberto Grassi¹, Davide Profaizer¹, Marco Valerio Rossi-Stacconi1

¹Technology Transfer Centre, Fondazione Edmund Mach, via Edmund Mach 1, 38010 San Michele all'Adige Trento, Italy

²Research and Innovation Centre, Fondazione Edmund Mach, via Edmund Mach 1, 38010 San Michele all'Adige Trento, Italy

In September 2009 in Trentino, for the first time in Europe economically important damage by Drosophila suzukii on soft fruits was reported. The consequent increasing use of insecticides, augmented the pesticide residues on the harvested fruits and jeopardised the results obtained with IPM on soft fruits. Development of alternative control methods appeared urgent to ensure an economic future for the concerned fruit industry and researchers and technicians of Fondazione Edmund Mach responded rapidly to this new threat. We considered that possible solutions would only arise from a coordinated and international network of diverse expertise, from molecular biology and neurophysiology to pest management techniques, aiming at understanding the fundamental aspects of the ecology of this pest. Accordingly, we have determined the genome sequence of Italian D. suzukii in order to assist both basic and applied research and to provide information about genes involved in processes such as intra- and inter-specific communication and overwintering. The mechanisms of fruit recognition and selection mediated by the olfactory and/or gustatory systems are being investigated. The population dynamics in different agroecosystems have been followed taking into consideration the population bottleneck after the winter reproductive diapause. Development, comparison and selection of the most reliable attractants have been performed to provide the growers with effective tools to track fly activity over time and properly time control measures. Mass trapping, physical crop protection by using anti-insect nets as well as botanicals and synthetic insecticides are under experimental evaluation in order to set up sustainable control strategies for the local soft fruit industry. Because of the legal preclusion from developing classical biocontrol, several indigenous parasitoids of larvae and pupae are under investigation as possible biocontrol agents in the framework of augmentative biocontrol. Pros and cons of the present control strategies will be discussed as well as the future directions of research on pest management of D. suzukii.

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CONTACT MAIL: claudio.ioriatti@fmach.it





rento, 7-9 November 2016



Biological control of spotted wing Drosophila by using Italian resident parasitoids: laboratory and field experiences

<u>Marco Valerio Rossi-Stacconi</u>^{1,2}, Aurore Panel³, Nuray Baser³, Tommaso Pantezzi¹, Claudio Ioriatti¹, Gianfranco Anfora²

¹Technological Transfer Centre, Fondazione Edmund Mach, via Edmund Mach 1, 38010 San Michele all'Adige Trento, Italy

²Research and Innovation Centre, Fondazione Edmund Mach, via Edmund Mach 1, 38010 San Michele all'Adige Trento, Italy

³Plant Protection in Organic Agriculture, Mediterranean Agronomic Institute of Bari (CIHEAM), Italy

Drosophila suzukii Matsumura, or Spotted Wing Drosophila (SWD), is a highly polyphagous invasive pest which has recently invaded Europe and Americas. Its huge economic impact is due, in part, to the absence of specialized natural enemies that suppress population outbreaks. Here we present a summary of the results of a three-year research on the possible role of indigenous Italian resident parasitoids as biological control agents for SWD.A series of laboratory experiments and extensive field trials was performed in order to evaluate the parasitization efficacy of potential SWD parasitoids and to acquire information on their developmental parameters, longevity, lifetime fecundity and host preference. We tested a total of five species: three natural occurring parasitoids, Leptopilina heterotoma Thomson, Pachycrepoideus vindemiae Rondani and Trichopria drosophilae Perkins, and two commercially available parasitoids, Spalangia cameroni Perkins and Nasonia vitripennis Ashmead. The two commercially available parasitoids were not able to develop on D. suzukii, whereas all the three natural occurring parasitoids disclosed a variable rate of parasitism. On the base of the laboratory experiments, the most effective parasitoid species, T. drosophilae, was chosen to perform semi-field and open field trials. The firsts were carried out in an isolated greenhouse, on artificially infested raspberries, while the seconds were performed in various orchards, on different susceptible crops. The parasitization rate was variable depending on the cropping system and environmental factors. However, our results open the way for a strategic use of *T. drosophilae* against SWD in the early season, in order to intercept the transfers of the pest from natural post-overwintering areas to the orchards. This study contributes to the development of biological control strategies of the pest which may become an important management tool in the near future for an area-wide control of SWD.

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CONTACT MAIL: valerio.rossi@fmach.it





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Models of *Drosophila suzukii* population dynamics

<u>Andrea Pugliese</u>¹, Souvik Bhattacharya¹, Ferdinand Pfab¹, Gianfranco Anfora², Marco Valerio Rossi-Stacconi^{2,3}, Alberto Grassi³

I will start with an overview of how to use laboratory data to obtain estimates of how demographic parameters of *Drosophila suzukii* depend on temperature and humidity. Population projections based solely on these and observed field temperatures will be shown, elucidating differences among years and sites. In order to apply the model to field data, it is necessary to consider at least larval competition and attractiveness of traps, both related to seasonal availability of fruits. I will show fitting to some trapping data of 2014-15. An interesting result is that data from traps set in parks and woodlands at low elevation are consistent with models only if it is assumed that large migrations occur to and from orchards at higher elevation; preliminary results from mark-recapture experiments indeed support the assumption. Finally, I will show some results from a simplified model that includes also a parasitoid species in order to explore potential effects of parasitoid release.

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CONTACT MAIL: andrea.pugliese@unitn.it

¹Department of Mathematics, University of Trento, Italy

²Research and Innovation Centre, Fondazione Edmund Mach, via Edmund Mach 1, San Michele all'Adige Trento, Italy

³Technology Transfer Centre, Fondazione Edmund Mach, via Edmund Mach 1, San Michele all'Adige Trento, Italy





FACING THE INVASION OF ALIEN ARTHROPODS SPECIES

ecology, modelling and control of their economic impact and public health implication **Trento, 7-9 November 2016**



Cost-benefit analysis for controlling *Drosophila suzukii* spread and infestation of soft fruits in Northern Italy

Emanuele Del Fava¹, Claudio Ioriatti², Alessia Melegaro^{1,3}

¹Carlo F. Dondena Centre for Research on Social Dynamics and Public Policy, Bocconi University, Milan, Italy

²Technology Transfer Centre and Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Trento, Italy

³Department of Policy Analysis and Public Management, Bocconi University, Milan, Italy

The objective of this work is to assess the economic impact of D. suzukii (or spotted-wing drosophila, SWD) infestation on the production of soft fruits in the province of Trento, Italy (also known as Trentino). SWD was firstly detected in Trentino in 2009 and the largest damage was reported in 2011, when no control strategy was in use. Since 2012, an integrated pest management (IPM) strategy was implemented, consisting of mass trap monitoring, field sanitation, and pesticide application. More recently, exclusion netting was also introduced in the area and proved to be more effective against SWD infestation. We performed a partial budget impact analysis, within the cost-benefit analysis (CBA) framework, to compare IPM and exclusion netting against each other and with respect to no intervention. This work innovates along three main directions: (i) using the CBA approach, we managed to compare the different strategies in terms of financial profitability for the whole soft fruit industry of Trentino; (ii) we also considered indirect societal costs (human health costs from use of pesticides) and benefits (positive externalities of control strategy from crop to crop); (iii) we assessed the robustness of our results to changes in parameters' values through a probabilistic sensitivity analysis using Bayesian MCMC methods. Overall, model results show that the economic impact of SWD pest in the absence of any control strategy corresponds to 8.5% of the potential revenues. This value decreases to 7.2% when implementing an IPM strategy, and it plummets to 4.5% when adopting exclusion netting. In other words, despite being the most expensive intervention, exclusion netting robustly emerges as a cost-beneficial strategy when compared to both IPM and to no intervention. This is valid also when exclusion netting is combined with mass trapping, which is the second most expensive interventions, with an overall probability of being cost-beneficial equal to 96% and 98% against IPM and no intervention, respectively. Although this result is not so strong when considering single crops, positive externalities may arise deriving from the application of the control strategy also to less affected crops, thus decreasing the overall presence of SWD in the area.

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CONTACT MAIL: emanuele.delfava@unibocconi.it





FACING THE INVASION OF ALIEN ARTHROPODS SPECIES

ecology, modelling and control of their economic impact and public health implication **Trento, 7-9 November 2016**



Development of the sterile insect technique to manage confined populations of Drosophila suzukii

<u>Fabiana Sassù</u>^{1,2}, Silvana Caravantes², Gustavo Taret², Katerina Nikolouli^{1,2}, Kostas Bourtzis², Rui Pereira³, Christian Stauffer¹, Mark Vreysen², Carlos Caceres²

¹Institute of Forest Entomology, Forest Pathology and Forest Protection, BOKU, University of Natural Resources and Applied Life Sciences, 1190 Vienna, Austria

²FAO/IAEA, Division of Nuclear Techniques in Food and Agriculture, A-2444, Seibersdorf, Austria
³FAO/IAEA, Division of Nuclear Techniques in Food and Agriculture, A-1400, Vienna International
Centre, Vienna, Austria

The Spotted Wing Drosophila fly (SWD), Drosophila suzukii (Diptera: Drosophilidae) is a member of the D. melanogaster group and is a destructive insect pest of fruit crops. Although most of its closely related sibling species are not fruit pests, the serrated ovipositor of the SWD permits ovipositing in undamaged fresh fruits. The invasion of SWD in Europe and America has a severe impact on the economy in view of its polyphagous habit, remarkable reproductive potential and fast development. As is the case with most insect pests of food in the world, the SWD is mainly controlled using insecticides. The use of insecticides is a concern of society and therefore farmers are demanding environmentally friendly alternatives to control this pest. The Sterile Insect Technique (SIT) is a promising approach that might be used to fight this invasive pest in confined areas such as greenhouses. Generally, males are mass-reared, sterilized by exposure to ionizing irradiation and released in the target areas where they compete with fertile wild males for wild females. Mating of a sterile male with wild female results in no offspring which eventually will reduce the pest population and the infestation. The SIT can only be applied efficiently against SWD when the insect can be adapted to laboratory conditions and protocols are available for their cost effective mass-rearing. In addition, protocols for quality control and mass-production of eggs, larvae, pupae, need to be developed as well as protocols for sterilization without compromising the quality and performance of the released sterile insects in the field. The goal of our project is to develop an efficient protocol for mass-rearing and sterilization of D. suzukii and to validate, initially in field cages, the potential of SIT, for the management of SWD in greenhouses. The success of this research will provide an innovative practical and biological solution to help address the threat of D. suzukii.

CONTACT MAIL: fabiana.sassu@gmail.com







Phylogenetic incongruences indicate a complex evolutionary scenario for the biocontrol agent *Wolbachia*

Francesco Drago, Omar Rota-Stabelli

Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

Wolbachia are maternally inherited intracellular endosymbionts bacteria, commonly found in arthropods and nematodes. Because of its ability to manipulate host's reproduction and influence the transmissibility of harbored pathogens, Wolbachia is a promising biological tool to control invasive pests. Various aspects of Wolbachia biology are still widely unexplored, particularly its evolution and its host's transfers: this knowledge is an important prerequisite to properly define control strategies. To further understand the evolution of Wolbachia we compare the phylogenetic signals of two independent data sets: a gene-rich phylogenomic data set and a taxonrich MLST data set. We first show that the two data sets provide incongruent topologies, and that the MLST genes significantly reject the phylogenomic topology: we suggest that this inconsistency may be explained by recombination or by strains misassignment due to undetected multiple infections. We then show that incongruences are also present within genes of the phylogenomic data set: this indicates either a complex evolutionary pattern involving recombination or misassembly due to multiple infections. We finally present a first effort to date the tree of Wolbachia using relaxed clock and previously proposed mutation rates. Our results indicate how recombination and/or multiple infections are clear sources of systematic error in Wolbachia phylogeny: this should be taken into account when inferring evolutionary scenarios and when typing new Wolbachia strains.

CONTACT MAIL: fradrag@gmail.com





ecology, modelling and control of their economic impact and public health implication. 7-9 November 2016



Genetic, evolutionary, symbiotic, and phenotypic variations between European and American strains of *D. suzukii*: implications for biocontrol and management studies

<u>Omar Rota-Stabelli</u>¹, Rupinder Kaur^{1,2}, Gabriella Tait^{1,3}, Stefanos Siozos^{1,4}, Marco Valerio Rossi-Stacconi⁵, Lino Ometto¹, Gianfranco Anfora¹

Interpreting the phenotypic value of intraspecific variation is key step to understand invasive species biology and effectively define their management. Here we combine genomic, evolutionary and physiological evidences to support a divergent origin of European and American populations of *D. suzukii*. Population genetics analyses reveal the presence of only two haplotype groups in south Europe, while North America is characterized by a larger genetic diversity. Evolutionary genomics further show that the two sequenced genomes (Italian and Californian) belong to distinct genetic backgrounds, have been subjected to different evolutionary forces, and have diversified hundreds of thousands of years ago in their native Asian areal. Using a novel typing system, we also found that European and American populations carry slightly distinct variants of *Wolbachia*, which may explain the different prevalence of this symbiont in the two continents. Finally, phenotypic studies show that Italian and Oregon populations are characterized by different fitness parameter rate and susceptibility to parasitoids. We advocate that these differences should be taken into account in management practices, particularly when findings obtained on one population are applied to another one, particularly if from a different continent.

CONTACT MAIL: omar.rota@fmach.it

¹Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

²Center of Anatomy and Cell Biology, Medical University of Vienna, Austria

³Department of Agricultural and Environmental Sciences, University of Udine, Udine, Italy

⁴Institute of Integrative Biology, University of Liverpool, UK

⁵Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige(TN), Italy





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Reconstruction the colonization history and the population dynamics of *Drosophila* suzukii in Italy

<u>Gabriella Tait</u>^{1,2}, Alberto Grassi³, Fabiana Sassù^{1,4,5}, Angela Gottardello³, Lino Ometto¹, Valerio Mazzoni¹, Gloria Antonini⁵, Omar Rota-Stabelli¹, Gianfranco Anfora¹

¹Research and Innovation Centre, Edmund Mach Foundation, San Michele all' Adige, Trento, Italy ²Department of Agricultural and Environmental Sciences, University of Udine, Udine, Italy ³Technology Transfer Centre, Edmund Mach Foundation, San Michele all'Adige, Trento, Italy ⁴Division of Nuclear Techniques in Food and Agriculture, FAO/IAEA, Wien, Wien, Austria ⁵Department of Biology and Biotechnologies Charles Darwin, Sapienza University of Rome, Italy

Drosophila suzukii, an insect species native to South-Eastern Asia, in the last years has invaded both Americas and Europe, and thanks to its serrated ovipositor and high dispersal capability, it has become one of the most destructive pest of soft fruits. In spite of its economic relevance, little is known regarding its gene flow and seasonal movement patterns. Therefore, in order to increase the knowledge concerning these aspects, two different experiments have been carried out: the first one inquires the capacity of *D. Suzukii* to move along a large-scale altitudinal gradient across different periods of season, the second one focuses on the genetic population structure of D.suzukii in Italy. For studying the movement pattern we selected a transect ranging from about 500to 1400 m a.s.l. altitude within woodlands along Valsugana and Mocheni Valleys (Trento Province, Italy), animportant soft fruit growing area with high D. suzukii population density. A combined marking-recapture and population genetics approach has been adopted. The markrecapture procedure was based on the use of chicken egg albumin as protein marker. Twice a week a solution of 10% chicken egg albumin was used for marking both vegetation and wild D. suzukii adults in the surroundings of the center of each study area. The presence of chicken egg albumin in the samples was detected via anti-proteic-specific enzyme-linked immunoassorbent assays (ELISA). First results indicated that D. suzukii showed surprising high dispersal rates over relatively long distances. Correlation analyses between D. suzukii captures, temperature trends and food source availability over the season are underway. Concerning the genetic population structure of *D. suzukii* in Italy, we used 15 previously characterized Simple Sequence Repeat (SSR) markers to estimate genetic differentiation across the genome of flies sampled in different geographical areas across Italy. Previously population structure analysis revealed genetic homogeneity between most populations, with the exception of flies collected in Sicily and Apulia. This could be due to a different introduction bottleneck or a specific environmental selective pressure. Future experiments will involve a Landscape Shape Interpolation analysis to define spatial pattern of *D. suzukii* genetic diversity in Italy.

CONTACT MAIL: gabriella.tait@fmach.it





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Transcriptome profiling of adult Drosophila suzukii antennae

Cristina M. Crava¹, Fabiana Sassù^{2,3}, Gianfranco Anfora¹

¹Research and Innovation Centre, Edmund Mach Foundation, San Michele all' Adige, Trento, Italy ²Institute of Forest Entomology, Forest Pathology and Forest Protection, BOKU, University of Natural Resources and Applied Life Sciences, 1190 Vienna, Austria

Drosophila suzukii is an emergent pest in Western countries that is seriously challenging soft fruit production. New and environmentally friendly control strategies are urgently needed to control the menace, and methods based in odor-guided behaviors may be promising. To this purpose, a comprehensive understanding of the genetics components underlying chemosensory perception is fundamental. Here, we present antennal transcriptome databases for both *D. suzukii* males and females. We then used these resources to identify genes involved in olfaction (chemosensory receptors and odorant binding proteins) whose expression is biased in one of the two sexes. Our data contribute to understand olfactory perception in *D. suzukii* and lay the foundation for a broader understanding of stimuli that may drive female oviposition behavior.

CONTACT MAIL: maria.crava@fmach.it

³FAO/IAEA, Division of Nuclear Techniques in Food and Agriculture, A-2444, Seibersdorf, Austria





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The role of wild hosts, natural habitat and low temperature to promote *Drosophila* suzukii invasiveness in North-East Italy

Lorenzo Tonina, Andrea Battisti, Folco Giomi, Lorenzo Marini, Manuel Sancassani, Nicola Mori

DAFNAE – University of Padova - Legnaro (Pd), Italy

The invasive Drosophila suzukii Matsumura (Diptera: Drosophilidae) was reported for the first time in Italy in 2009 in Trentino-Alto Adige and one year later in Veneto region, where it is causing heavy damage on cherry, soft fruit, and grape. Among other features, the invasion success of D. suzukii is promoted by its generalist behaviour in relation to the host plants. Thus, characterizing the range of host plants in natural habitats across elevational gradients becomes essential to develop sustainable IPM strategies. To estimate adult population density and the source-sink relations between wild and cultivated habitats, field surveys were carried out in 20 vineyards in Valpolicella area (Veneto, Verona district) starting from June 2013 and using food traps. A collection of fruits of non-crop plants was also undertaken in both 2014 and 2015. In addition, parameters like elevation, temperature, and extension of forest cover around the collection point were measured. Results showed that 34 non-crop plants were reported as hosts of D. suzukii and that D. suzukii is significantly more abundant above 500 m, where temperature is lower and forest cover more extended. To assess the abundance of the fly in mountain areas in summer months, potential wild host fruits were sampled along two gradients of elevation to measure fly emergence under different temperature conditions. In addition, a natural temperature gradient in an opentop cave, covering the lower range of temperatures known for D. suzukii, was exploited to deploy laboratory stock colonies. In mountain areas, adults were obtained when the daily average temperature in the three preceding weeks was at least 11.1°C. Similar results were obtained with the laboratory colonies reared in the cave, where oviposition and development from egg to adult occurred above 11.6°C. These findings indicate that D. suzukii performs well at low temperatures and provide indications about the possible extension of distribution range in mountain areas with evident consequences on crops and wild host reproduction. It emerges the need to set up monitoring and control efforts of this new pest, especially in wooden high hills areas.

CONTACT MAIL: lorenzo.tonina@gmail.com





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Comparative study on ammonia and urea tolerance in *Drosophila melanogaster* and *Drosophila suzukii*

Virginia Belloni, Alessia Galeazzi¹, Giulia Bernini¹, Elisabetta Versace, Albrecht Haase^{1,2}

In the last decade, growing concern has turned on the invasive pest *Drosophila suzukii*, a serious agricultural and economical threat. Differently from other Drosophila species, females of D. suzukii lay eggs just under the skin of fresh fruits, through a serrated ovipositor. Therefore, larvae development and exposure to pathogens result in damage of a wide range of small and stone fruits. Understanding of the ecology and biology of this species is important to develop management strategies and successfully minimize its spread and impact. To date most of research has focused on D. suzukii adults, with little attention to the larvae, which are not only the direct threat to fruit but also the vehicle of perpetration of new generations. The more innocuous Drosophila melanogaster lay eggs in fermented fruits and larvae develop in a crowding environment characterized by accumulation of nitrogenous waste such as ammonia and, at lower extent, urea. Behavioral avoidance cannot prevent larvae exposure to environmental toxins, so physiological mechanisms evolved to cope with these compounds and their effects. While it is known how *D. melanogaster* respond to high concentrations of urea and ammonia, little is known on the potential effects on D. suzukii. We investigate the effect of different concentrations of these compounds on female fecundity and larval development in D. melanogaster and D. suzukii. Both females and larvae of D. suzukii showed a greater sensitivity to high concentration of urea and ammonia, with a drastic decrease in fecundity and egg viability. Adaptation to a different ecological niche, shifting from rotten fruit to fresh fruit as oviposition substrate, has allowed larvae to develop in a safer and healthier environment. However, metabolic adaptations to different food components and environment have probably resulted in less efficient detoxifying and excretory mechanisms. Further studies are ongoing to better understand the interaction between female fecundity and nitrogenous compounds. To shed light on those mechanisms in D. suzukii is a necessary step to plan effective strategies of sustainable pest control.

CONTACT MAIL: virginia.belloni@unitn.it

¹Center for Mind/Brain Sciences, University of Trento, Italy

²Department of Physics, University of Trento, Italy





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Monitoring of invasive arthropods in highway rest areas in Hungary

Balázs Kiss, Zsolt Kárpáti, Béla Molnár, Éva Szita

MTA Centre for Agricultural Research, Plant Protection Institute, Hungary

Highway rest areas offer an excellent opportunity for the monitoring of the spreading of different invasive species. In our project, we used Hungarian highways as transects, with 33 main sampling sites for a multiyear study. The survey focused on one hand on designated invasive species (e.g. Metcalfa pruinosa, Harmonia axyridis, Drosophila suzukii), and on the other hand on the characterisation of arthropod assemblages of the sites. Highway rest areas were demonstrated to have surprising species richness in many arthropod groups. For instance, in Bruchinae, Auchenorrhyncha, Orthoptera, Araneae, Heteroptera and Carabidae, 81%, 37%, 36%, 35%, 34% and 22% of the species known from Hungary were found in these habitats, respectively. More than 40 species were found for the first time in Hungary. Among them, spotted wing drosophila represents the most economic significance. In 2012 and 2013, only 38 SWD specimens were found in Hungary and they were found only in highway rest areas. In 2014 close to 10000 individuals were recorded in our project, and the species was also found in orchards. Our project has demonstrated different spreading scenarios at countrywide scale in different invasive species. For instance, in the citrus flatid planthopper, the distribution pattern of the species remained mosaic for several years after its first detection, due to the slow natural frontal spread combined with sporadic long distance transfers by human activity. In contrast, the distribution area of thespotted wing drosophila covered practically the whole territory of the country for the third year following its first detection. It can be concluded, that monitoring of highway rest areas is a useful tool for detecting new species in invasion and also for better understanding the mechanisms of spread of several arthropod species. (The project is supported by NKFI, project ID: 83829 and 119844).

CONTACT MAIL: kiss.balazs@agrar.mta.hu





KEYNOTE TALK

Advances in the knowledge of *D. suzukii* physiology and ecology

Vaughn Walton

Department of Horticulture, Oregon Wine Research Institute, Oregon State University, USA

Drosophila suzukii, Spotted Wing Drosophila (SWD) is a pest that has worldwide economic impact on susceptible fruit production. The impact of *D. suzukii* threatens the long-term sustainable production of these commercial fruits. The risk of *D. suzukii* fruit damage resulted in a significant increase in production inputs in all affected crops and production regions. *D. suzukii* is highly adaptable and can survive in a range of stresses including temperature and pesticides. We describe the stress factors under which *D. suzukii* can be found and how these adaptations result in ultimate population expansion. The physiological and ecological limitations of *D. suzukii* however can be used inmanagment techniques in a variety of production systems. We describe techniques that can be used in an integrated system to more effectively manage *D. suzukii*. Such techniques include refined population estimation, environmental manipulation, biological control and promising cultural control techniques. We present data that indicate the importance of biological control as a contributing factor for *D. suzukii* management.

CONTACT MAIL: vaughn.walton@oregonstate.edu



Trento, 7-9 November 2016





Author index

Accorsi Annalisa	30	Da Rold Graziana	38
Acutis Pier Luigi	30	Damiani Claudia	27
Ahrens Bodo	25	Dani Francesca Romana	17
Albert Céline	13	De Camilli Marina	30
Anfora Gianfranco12; 17; 41; 42	2; 43; 47; 48; 49	Del Fava Emanuele	44
Antonini Gloria	48	della Torre Alessandra	31; 32; 33; 36
Arcà Bruno	15	Dhimal Meghnath	25
Arnoldi Daniele	38; 39	Di Giulio Mario	29
Arthofer Wolfgang	9	Drago Andrea	38
Baldacchino Frederic Alexandre	38; 39	Drago Francesco	46
Baldini Marta	12	Durante Serena	30
Ballardini Marco	30	Engeler Lukas	28
Barbet-Massin Morgane	13; 18; 19	Failloux Anna-Bella	23
Baser Nuray	42	Favia Guido	27
Battisti Andrea	50	Ferretti Stefano	30
Bean Collin	14	Filipponi Federico	31
Bellard Céline	13	Fiorillo Carmine	15
Belloni Virginia	51	Flacio Eleonora	28
Bernini Giulia	51	Fontaine C	19
Bertolino Sandro	20	Fournier Alice	13; 19
Bhattacharya Souvik	43	Galeazzi Alessia	
Bianchi Cesare		Gasperi Giuliano	10; 11; 23
Bonizzoni Mariangela	35	Giomi Folco	50
Bortolotti Laura	17	Giovannozzi Matteo	29
Bourtzis Kostas	45	Gomulski Ludvik M	10
Bradshaw Corey J. A	13	Gottardello Angela	48
Bussola Francesca	38	Grassi Alberto	41; 43; 48
Caceres Carlos	45	Grieco Cristina	29
Capelli Gioia	38; 39	Groneberg David A	25
Cappa Federico	17	Guglielmino Carmela R	23
Caputo Beniamino	31; 32; 33; 36	Guidetti Roberto	12
Caravantes Silvana	45	Guidi Valeria	28
Cardellino Umberto	30	Haase Albrecht	51
Carlin Sara	38	Houghton Rupert	14
Casalone Cristina	30	Hudson Peter J	
Cervo Rita	17	Ioriatti Claudio	41; 42; 44
Chen Xiaoguang	35	Kárpáti Zsolt	52
Chiaranz Giorgio	30	Kaur Rupinder	47
Cini Alessandro	17	Kiss Balázs	52
Cobre Pietro		Knauß Dennis	34
Courchamp Franck	13; 18; 19	Kreß Aljoscha	24
Crava Cristina M		Kuch Ulrich	
Cremonesi Ilaria	30	Lambin Xavier	14
D'Alessandro Antonello	32	Laughton Robert	14



ecology, modelling and control of their economic impact and public health implication Trento, 7-9 November 2016





Laurino Daniela	20	Radaelli Maria Cristina	30	
Leroy Boris	13	Ravagnan Silvia	38	
Lioy Simone	20	Reuss Friederike	34	
Lombardo Fabrizio	15	Ribeiro José M	15	
Maistrello Lara	12	Riina Maria Vittoria	30	
Malacrida Anna R	10; 11; 23	Rizzoli Annapaola	38	
Mancini Maria Vittoria	27	Roberto Paolo	29	
Manica Mattia	31; 32; 33; 36	Roiz David	13	
Manino Aulo	20	Rome Quentin	in 18; 19	
Manni Mosè	23	Rosà Roberto		
Marcantonio Matteo	39	Rossi-Stacconi Marco Valerio	12; 41; 42; 43; 47	
Marconcini Michele	23	Rota-Stabelli Omar	26; 46; 47; 48	
Marini Lorenzo	50	Salles Jean-Michel	13; 18	
Mazzoni Valerio	12; 48	Salvemini Marco	15	
Melegaro Alessia	40; 44	Sancassani Manuel	50	
Merler Stefano	·	Sassù Fabiana	45; 48; 49	
Merli Daniele	11	Savini Grazia	10; 11	
Metz Markus		Schuler Hannes	•	
Mignone Walter		Scolari Francesca		
Molnár Béla		Serra Massimo	, ,	
Montarsi Fabrizio		Simard Frédéric		
Mori Nicola	•	Simoni Alekos		
Mosca Andrea		Siozos Stefanos		
Müller Pie		Stauffer Christian		
Müller Ruth		Suter Tobias	•	
Neteler Markus		Szita Éva		
Nikolouli Katerina		Tait Gabriella		
Nolan Tony		Taret Gustavo	,	
Ometto Lino		Tetzlaff Doerthe		
Oppold Ann-Marie		Tonina Lorenzo		
Panel Aurore		Tonolla Mauro		
Pantezzi Tommaso		Trentini Filippo		
Pautasso Alessandra		Turelli Michael		
Pepiciello Irene		Turillazzi Stefano		
Pereira Rui		Vavassori Laura		
Perna Mirko	_	Vega-Rua Anubis		
Petrocelli Irene		Verna Federica		
Pfab Ferdinand		Versace Elisabetta		
Pfenninger Markus		Villemant Claire		
Pichler Verena		Vreysen Mark	•	
		•		
Pinto Joao		Walton Vaughn Wilhelm Johannes		
Polajnar Jernej				
Poletti Piero		Xu Jiabao		
Profesior Davide		Yan Guiyun		
Profaizer Davide		Zeller Herve	22	
Pugliese Andrea	43			